

**ISHI Workshop on New Loci and Kits**

October 2, 2014 (Phoenix, Az)

**New Autosomal and Y-STR Loci and Kits:**

**Making Data Driven Decisions**

# Experience with PowerPlex Fusion

**Jeffrey Nye**

Michigan State Police



# PowerPlex Fusion



- Interest in looking at extended STR Loci panels
- At time decision was made to look at Fusion, we were using 3130 and 3130xl analyzers
  - Have since purchased 3500s
- Offender database of 300,000+ include Penta loci

# • INTERNAL VALIDATION

- Casework Section
- Studies completed include:
  - Precision
  - Sensitivity
  - Baseline noise evaluation
  - Contamination Assessment
  - Mock Casework
  - Mixtures
  - Concordance
  - NIST SRM

Validation at One Laboratory  
(Completed)

Performance Verification  
Studies at Two Casework  
Laboratories (Completed)

Training, Competency Testing  
and Planned Implementation  
System-wide (ongoing).

# Internal Validation

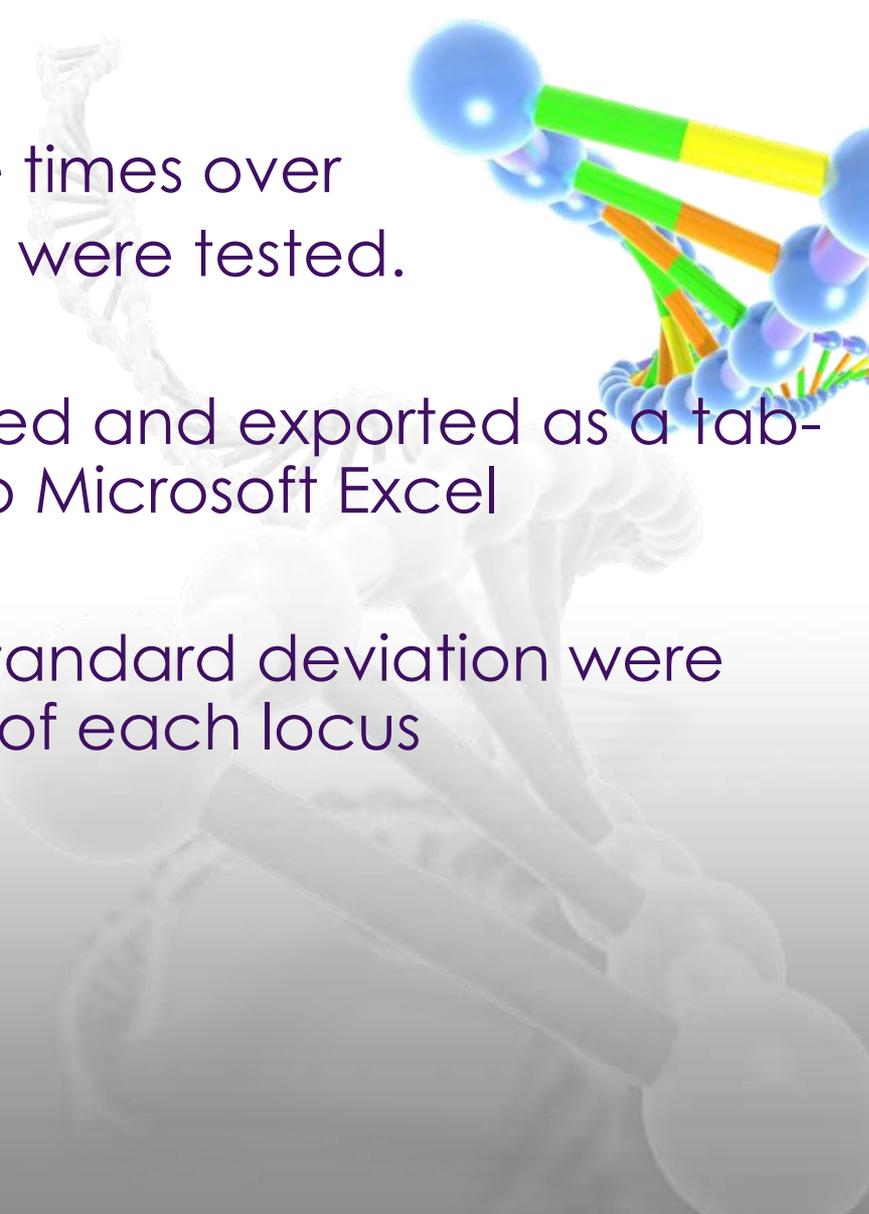
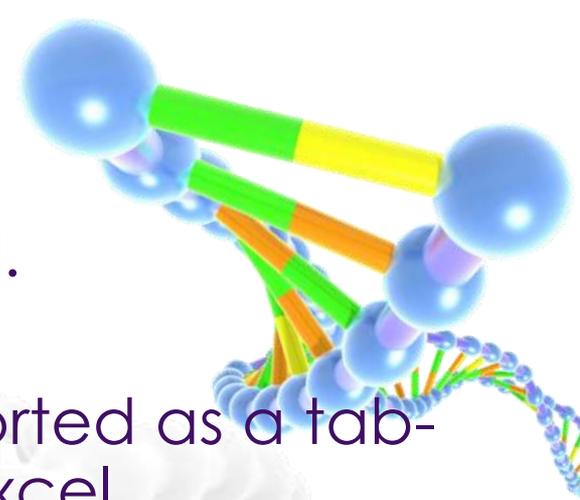


## Precision

Ladders were run multiple times over a few days. All capillaries were tested.

A table in GMID was created and exported as a tab-delimited file for import into Microsoft Excel

The average bp size and standard deviation were calculated for each allele of each locus



# Internal Validation



Precision

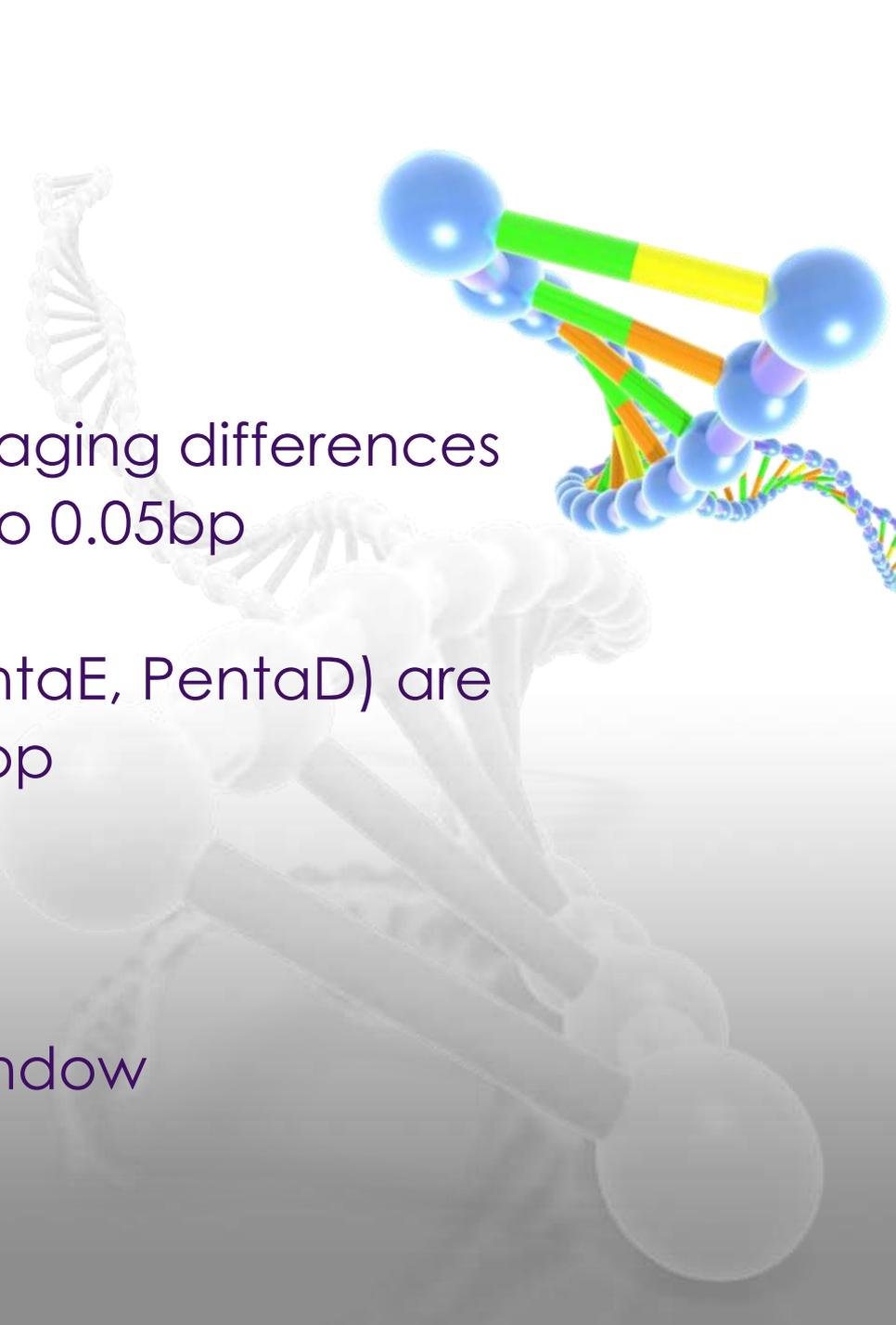
In general:

Smaller loci are averaging differences approximately 0.03 to 0.05bp

Larger loci (FGA, PentaE, PentaD) are averaging 0.08-0.09bp

3x Standard Deviation

Well below 0.5bp window

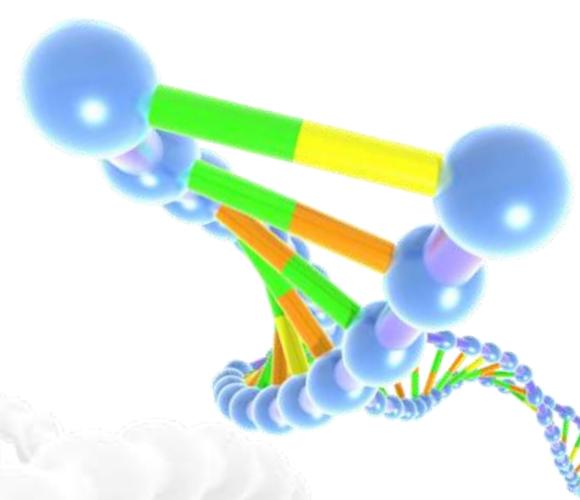


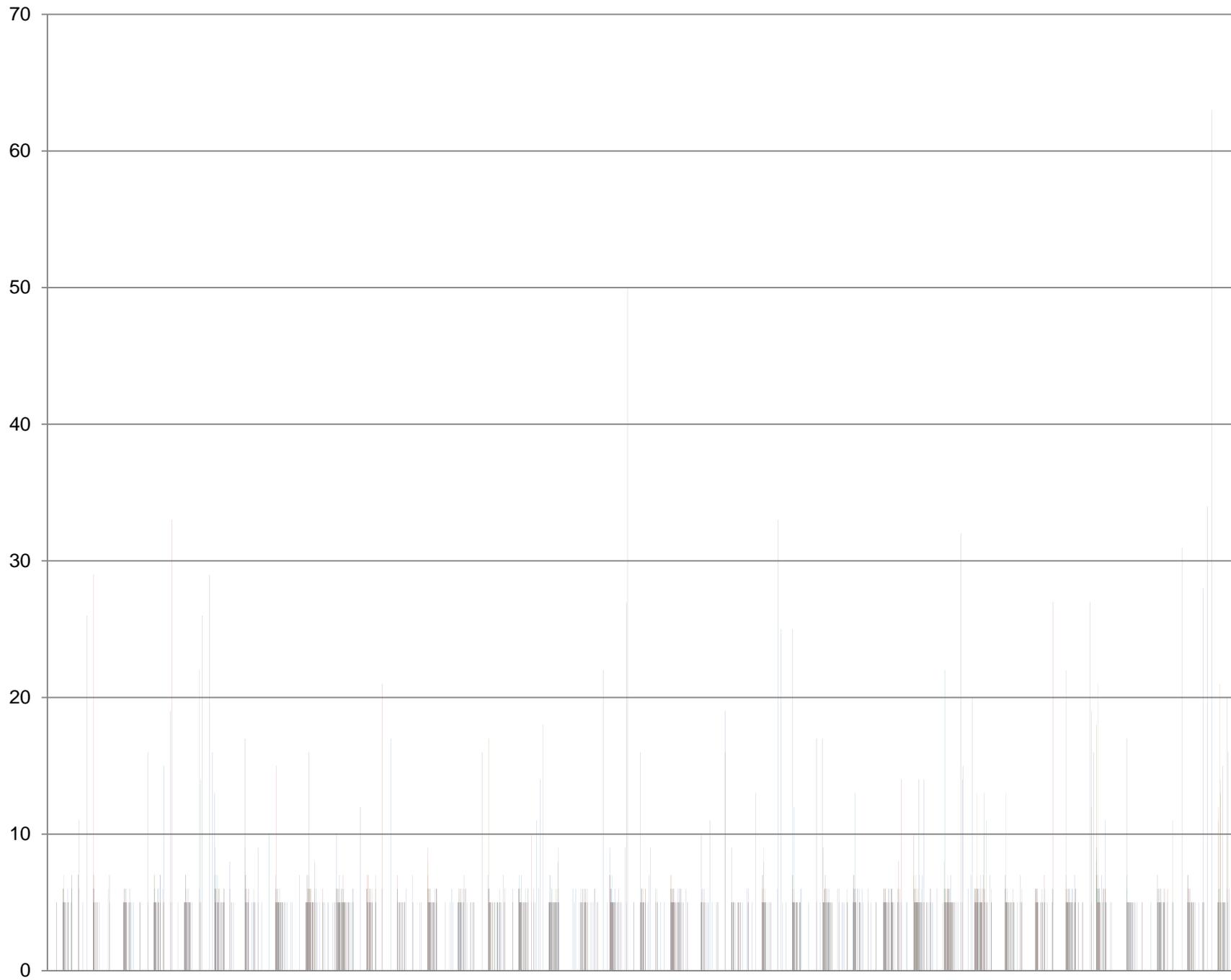
# Internal Validation



## Contamination Assessment and Baseline Noise Evaluation

40 Reagent Blanks were amplified

- Stain Extraction Blanks
  - Maxwell Extraction Blanks
  - Epithelial Extraction Blanks
  - Sperm Fraction Blanks
- 
- Run on 3130
  - Analyzed at 5 rfu
  - GMID table created and exported to Excel
- 
- Average Peak height was 5.8rfu +/- 3.2 rfu
- 
- A few peaks were labeled > 25 rfu
  - DNA TL option...AT 75 RFUs
- 
- 



# Internal Validation

## Sensitivity

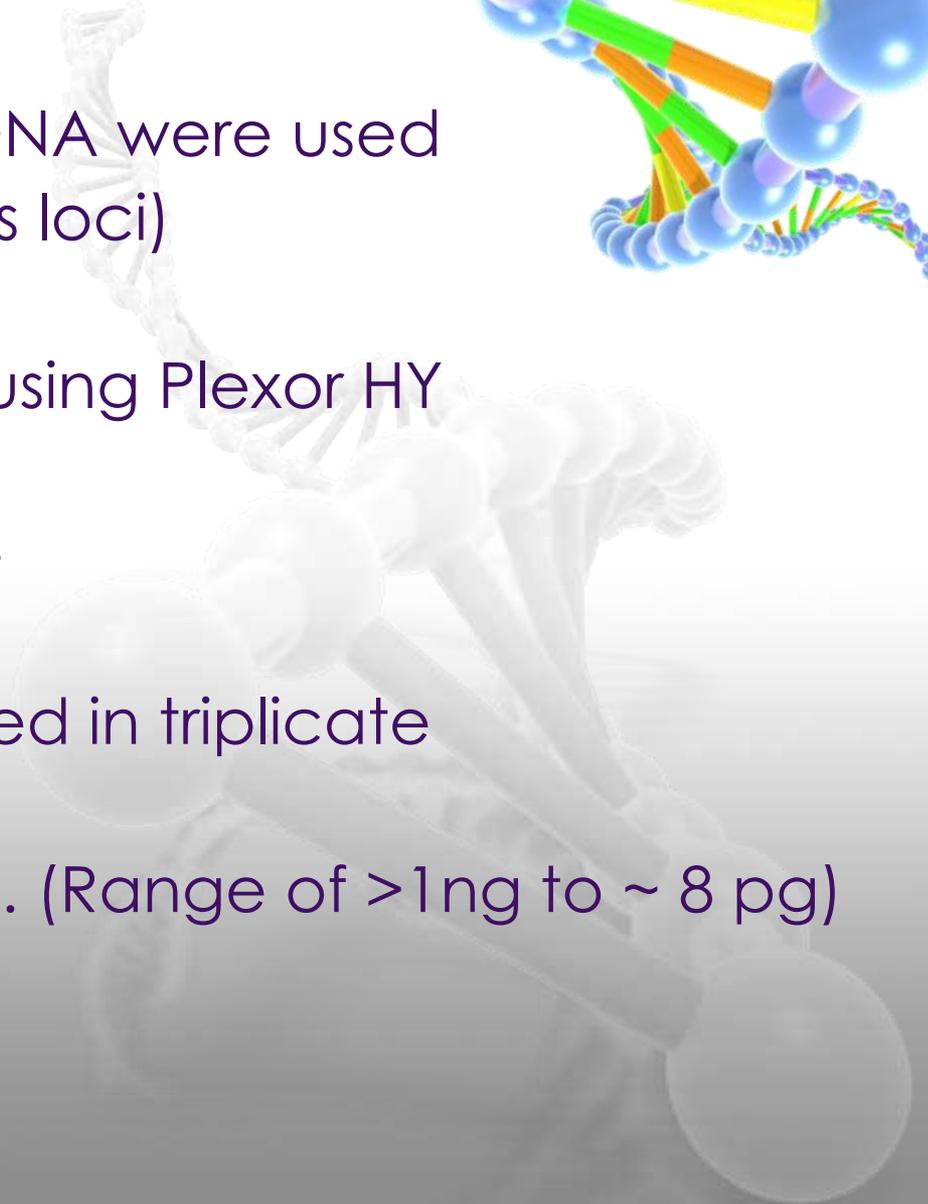
3 samples of extracted DNA were used  
(Many heterozygous loci)

Samples were quantified using Plexor HY

Serial dilutions were made

Each dilution was quantified in triplicate

Replicates were amplified. (Range of >1ng to ~ 8 pg)



# Internal Validation

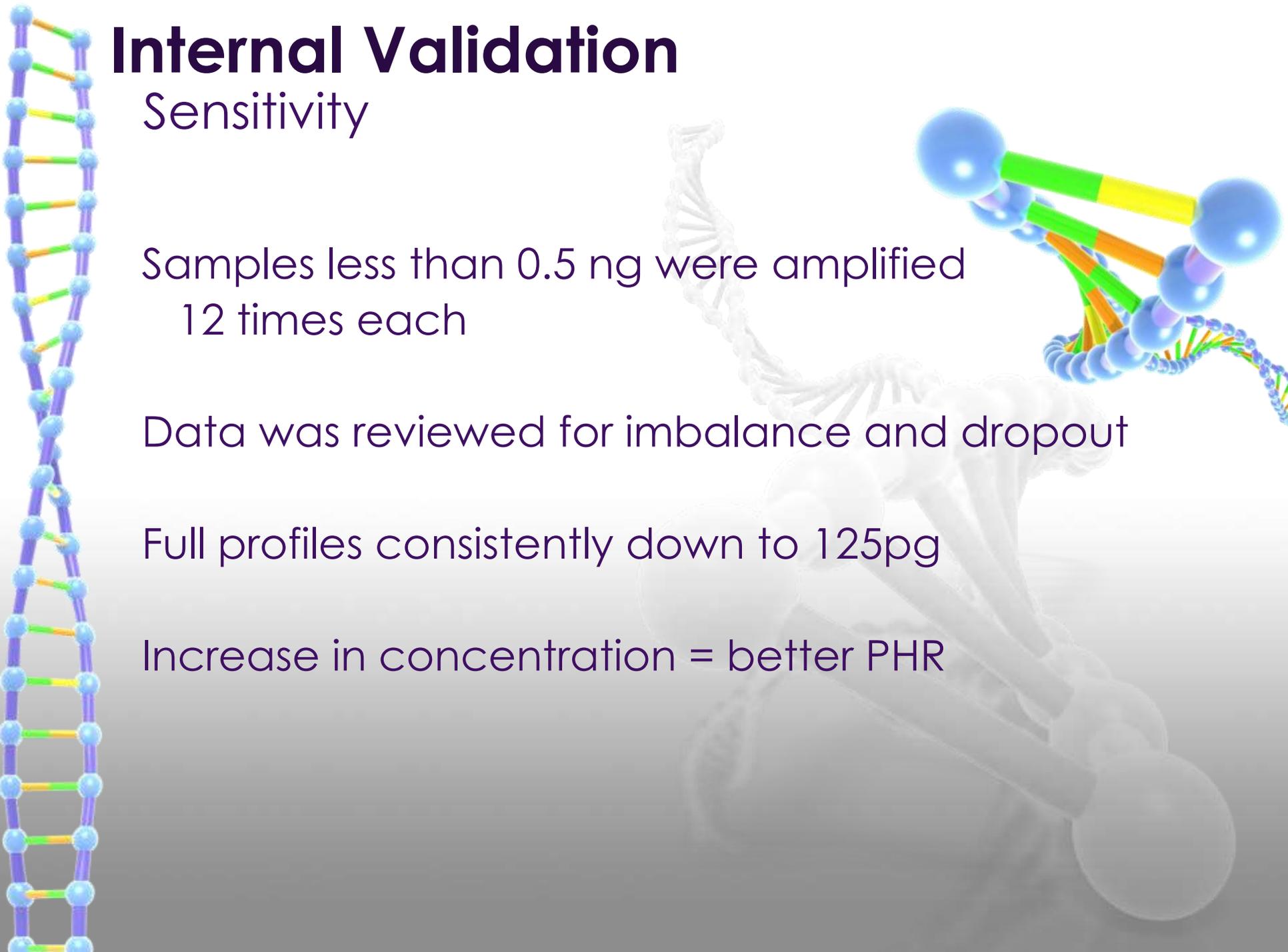
## Sensitivity

Samples less than 0.5 ng were amplified  
12 times each

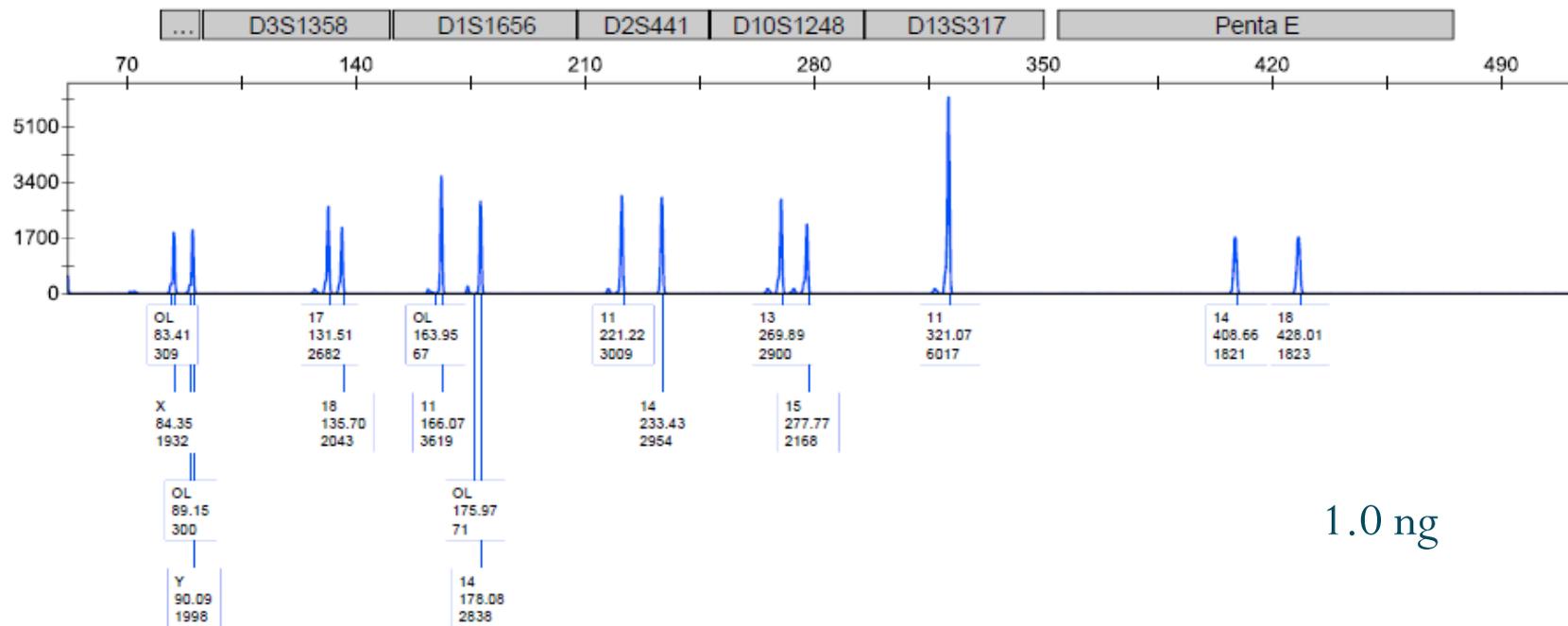
Data was reviewed for imbalance and dropout

Full profiles consistently down to 125pg

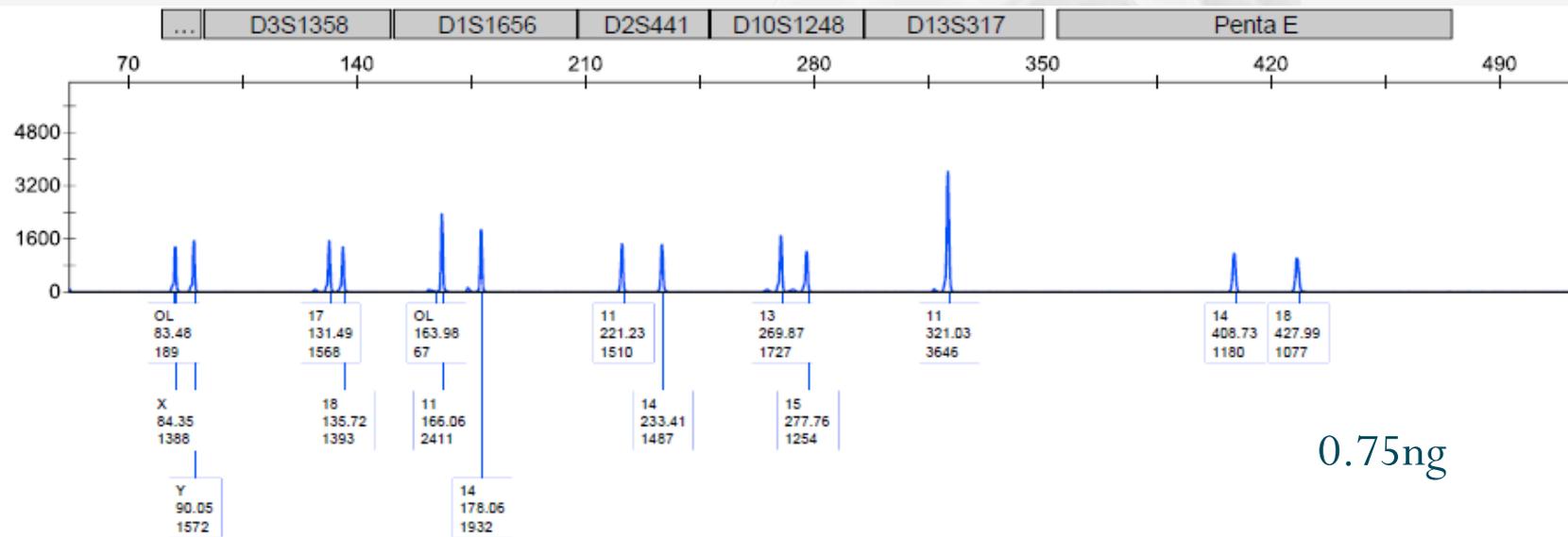
Increase in concentration = better PHR



# Internal Validation

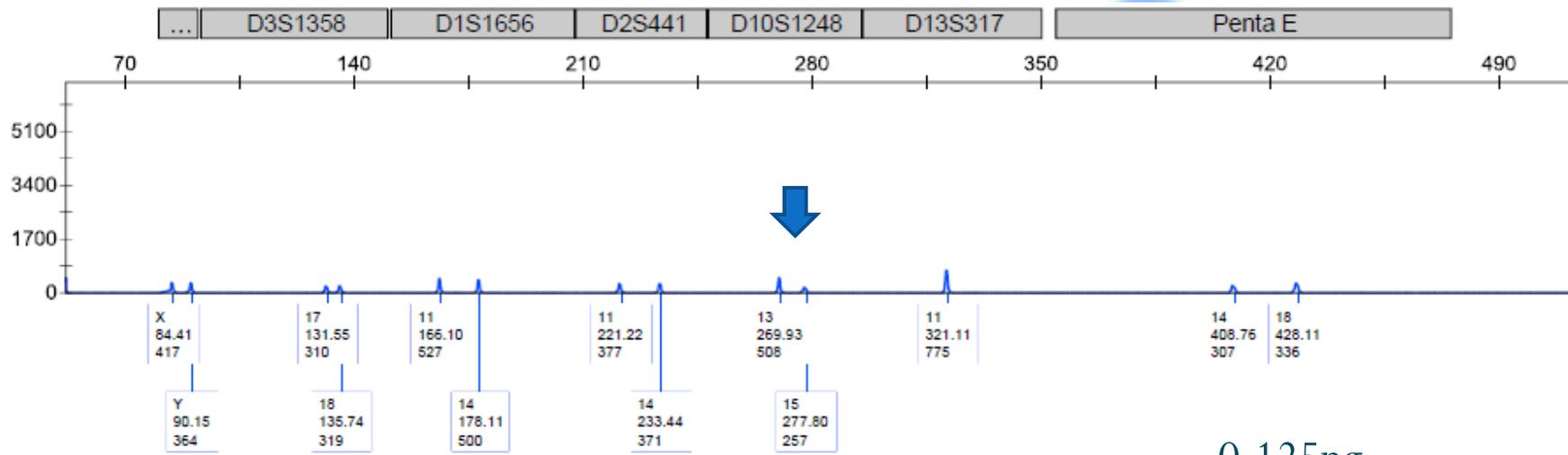


1.0 ng

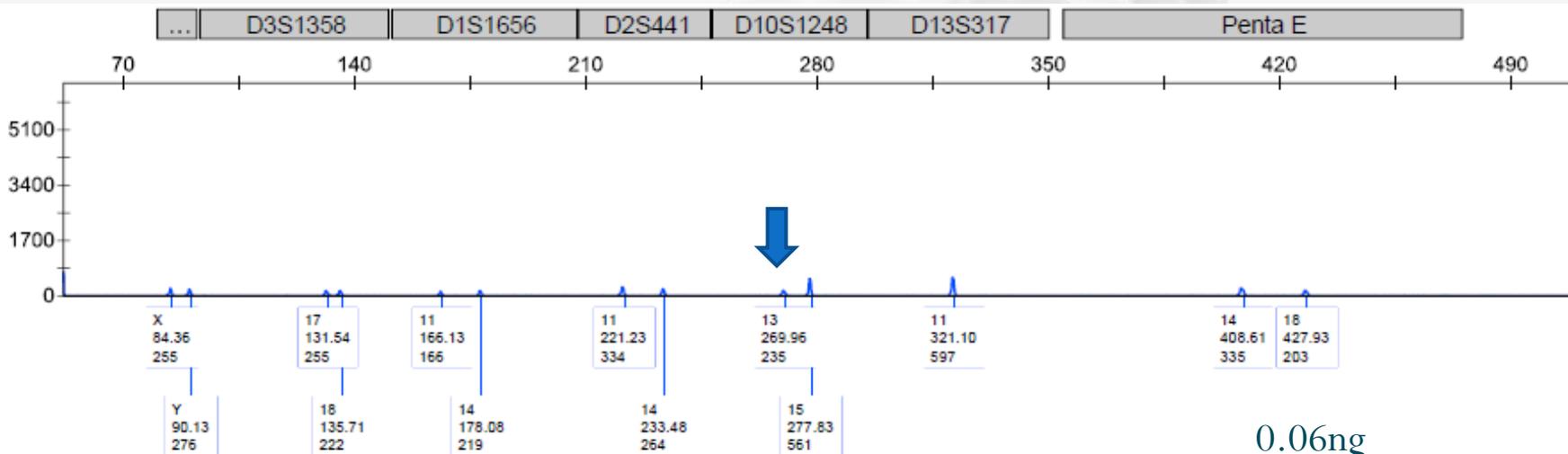


0.75ng

# Internal Validation

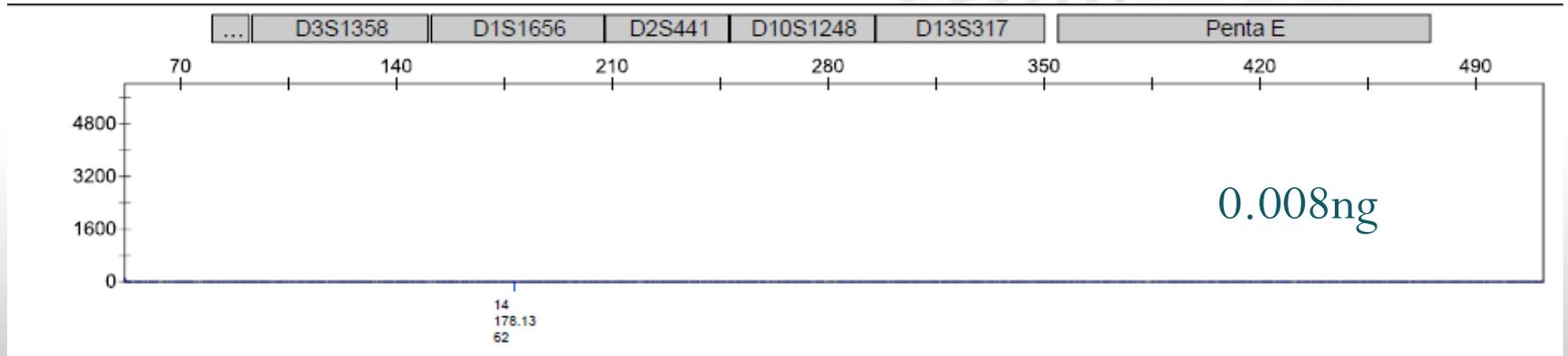
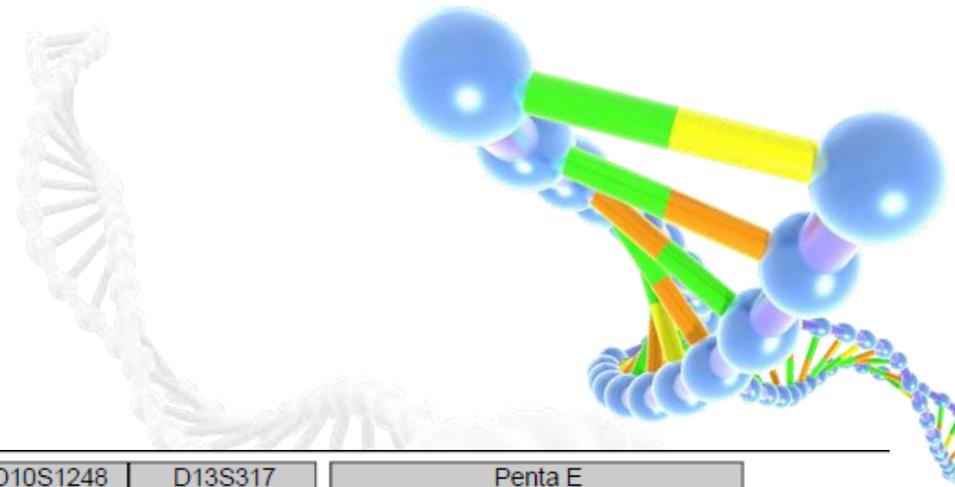


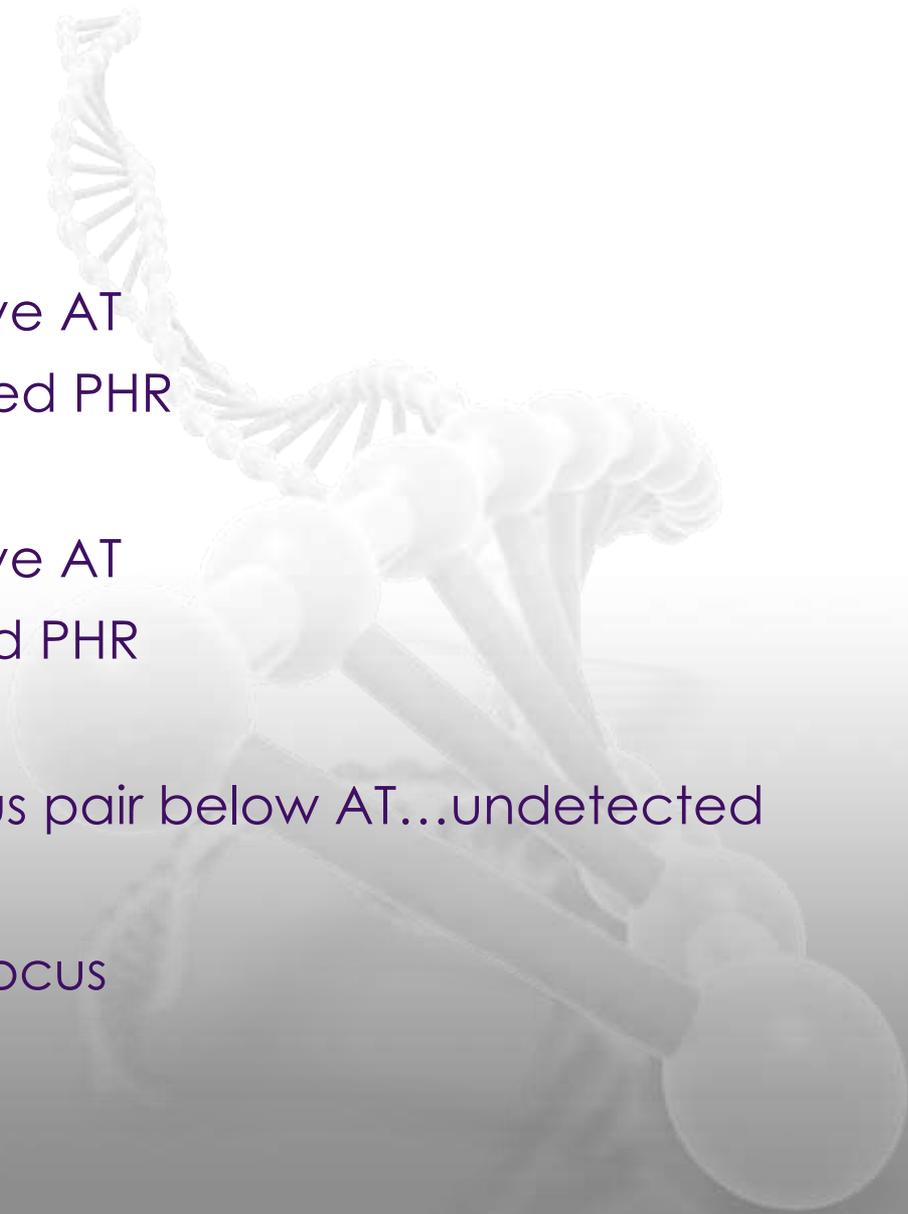
0.125ng



0.06ng

# Internal Validation



- 
- Green...good
    - Heterozygous alleles above AT
    - Balanced within established PHR
  - Yellow...not bad
    - Heterozygous alleles above AT
    - Balance under established PHR
  - Red...partial data
    - One allele of heterozygous pair below AT...undetected
  - Blue...total locus loss
    - No results at a particular locus







31 pg, 50% PHR, AT 75, ST 300



# Internal Validation

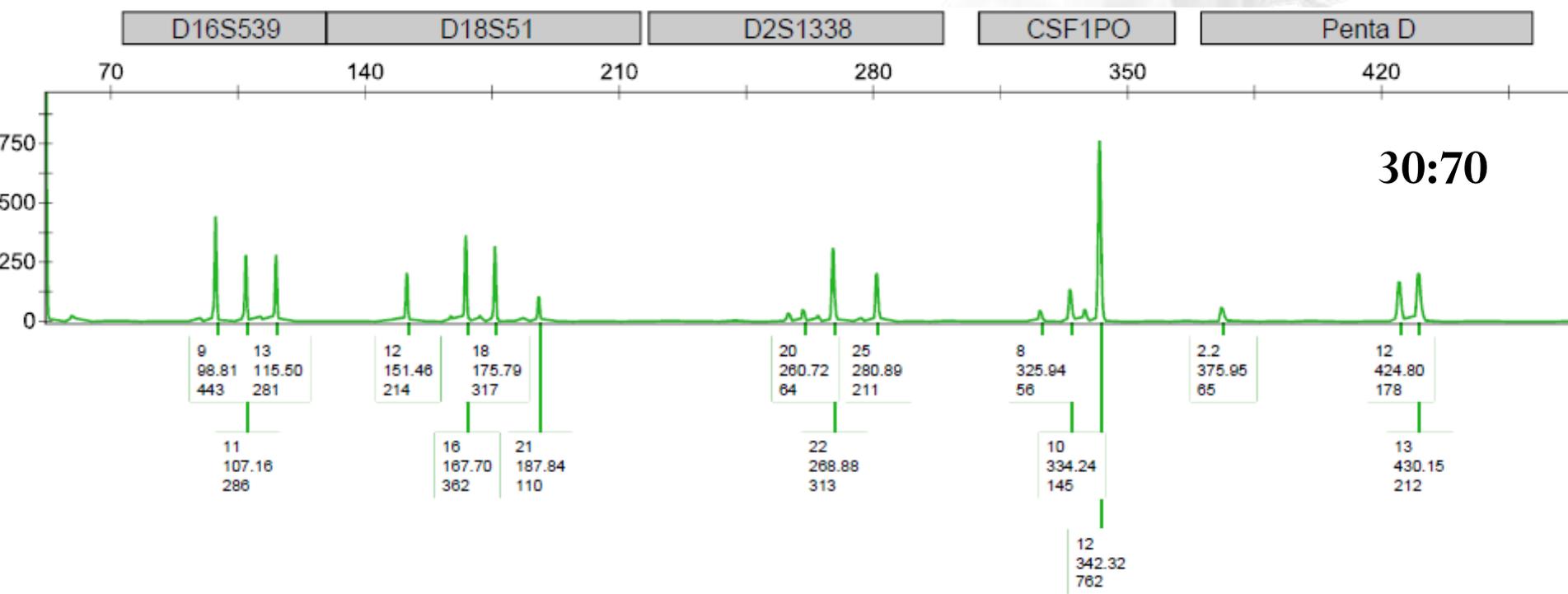
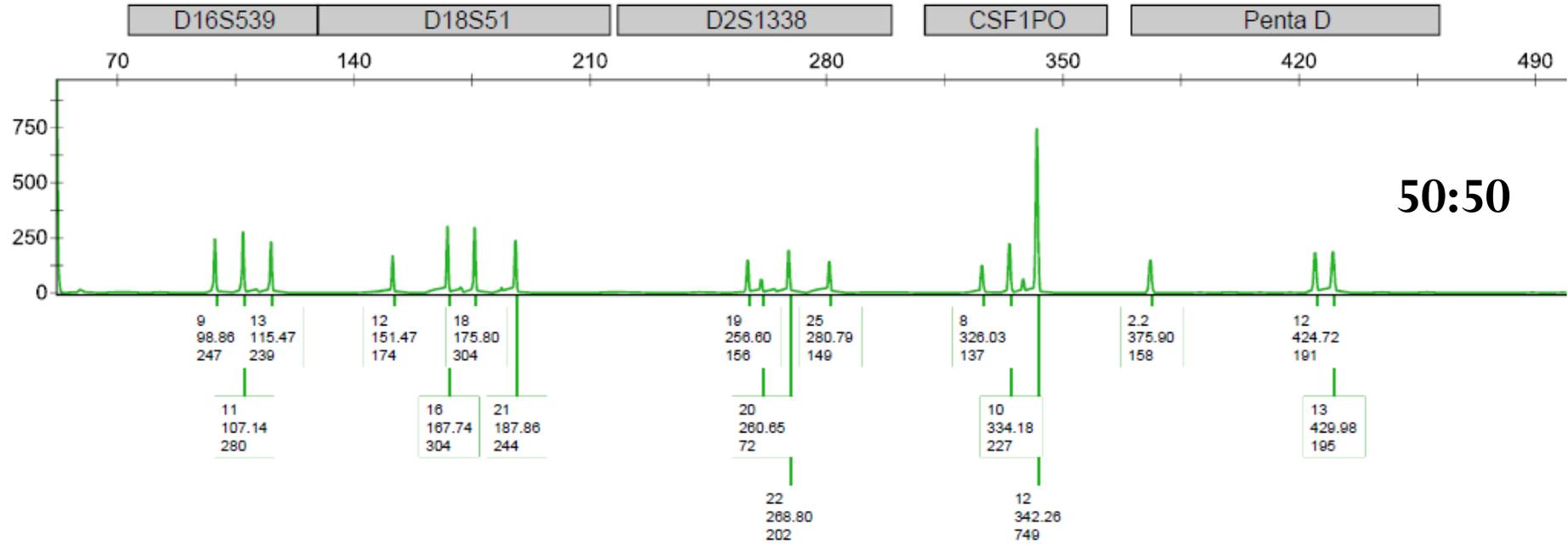
## Mixtures

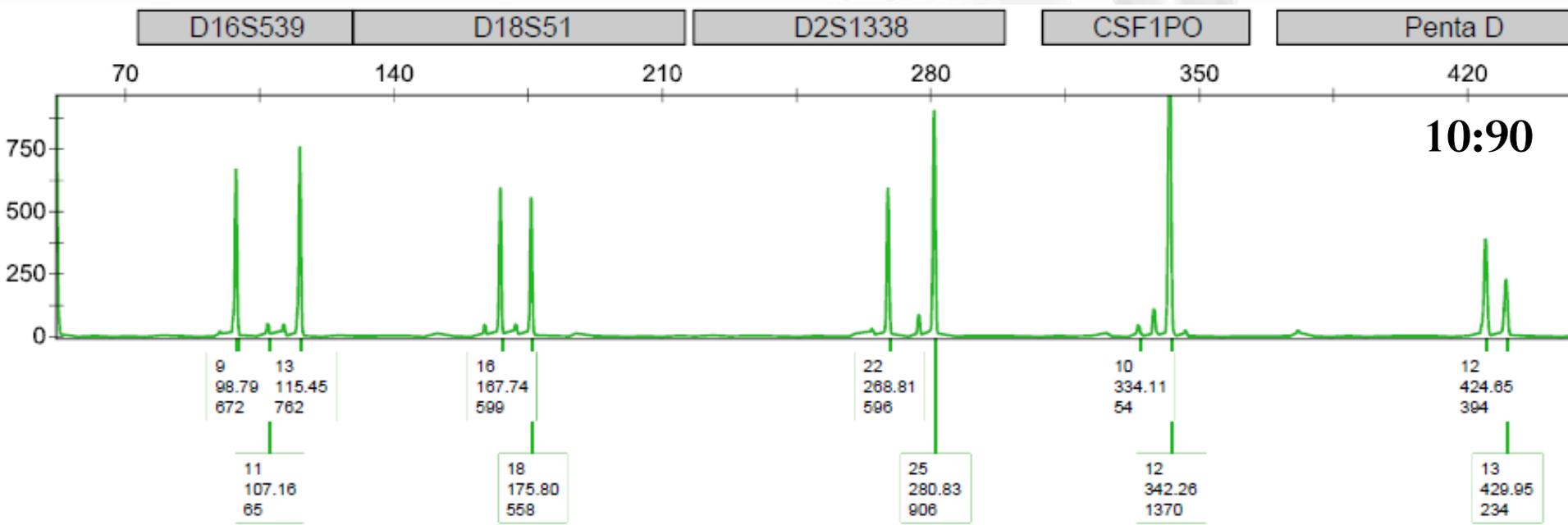
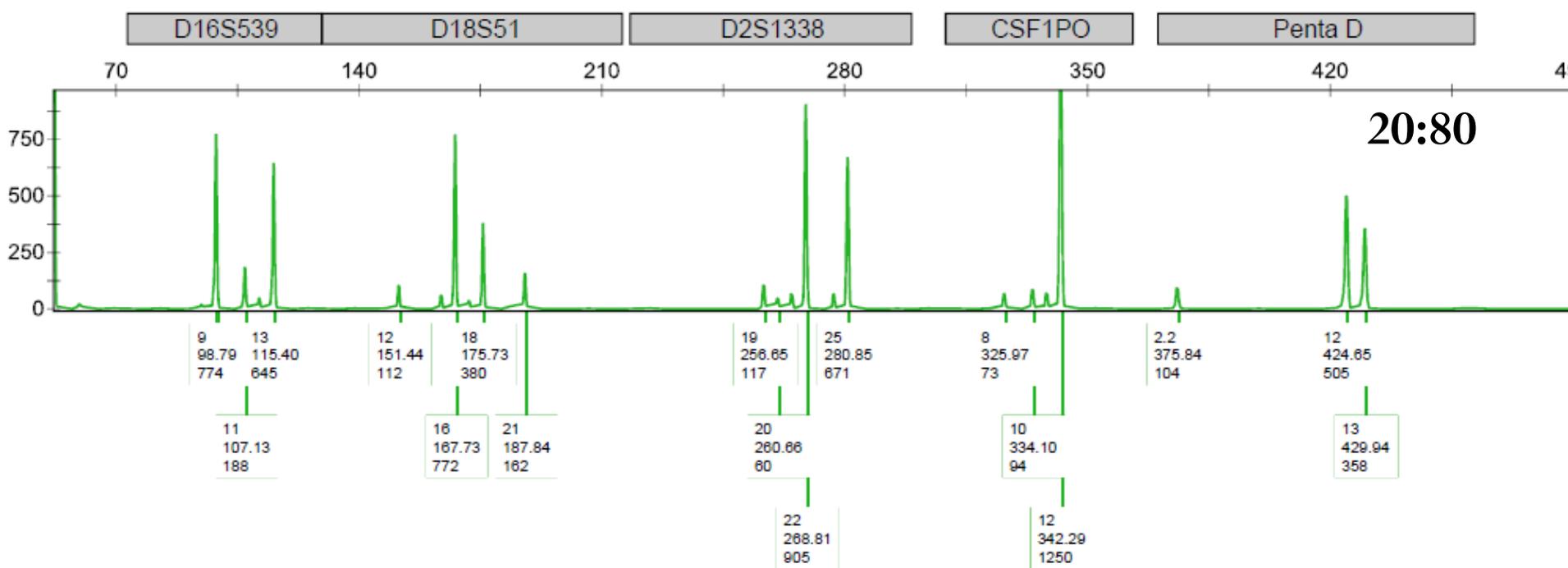
Mixtures prepared from extracted reference sample DNA

Ratios from 100:0 to 0:100 were prepared with an input template amount of 0.75ng

Amplified product was ran on a 3130 with a standard 5 second injection time

Minor donor detected at all mixture ratios





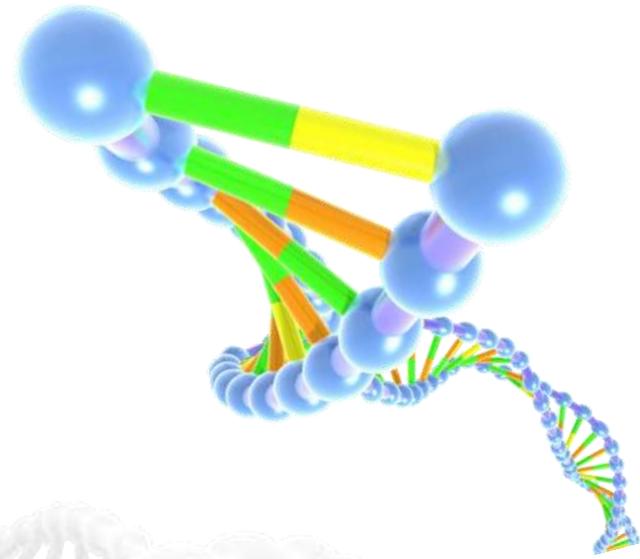
# Internal Validation

## Casework

Fortunate to be able to use actual casework samples

More than 40 cases have been analyzed with Fusion-  
only able to show a small portion here

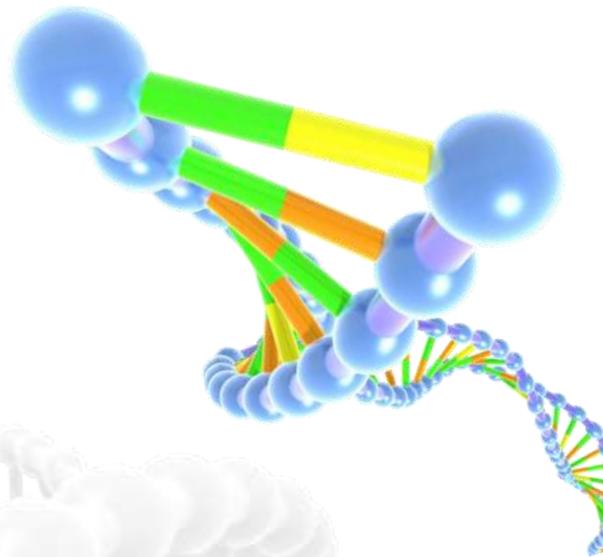
Concordant data overall

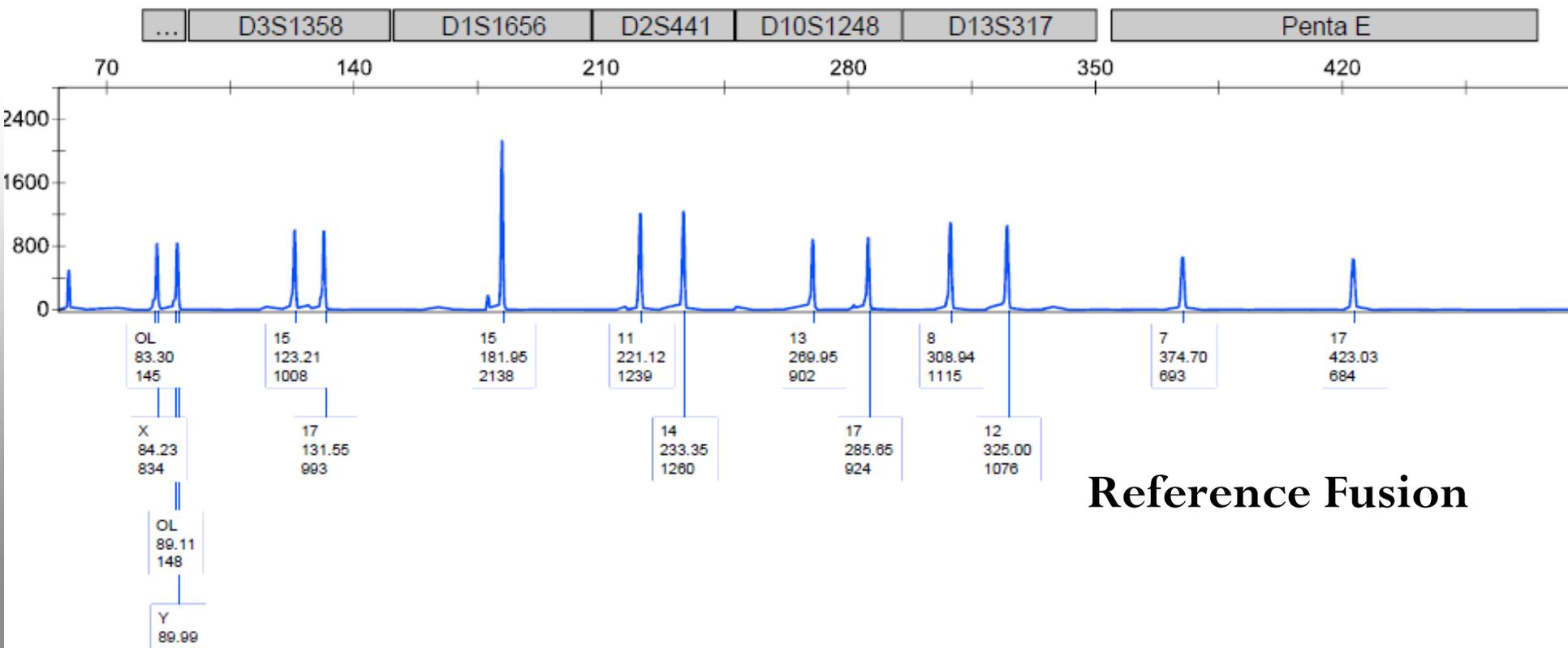
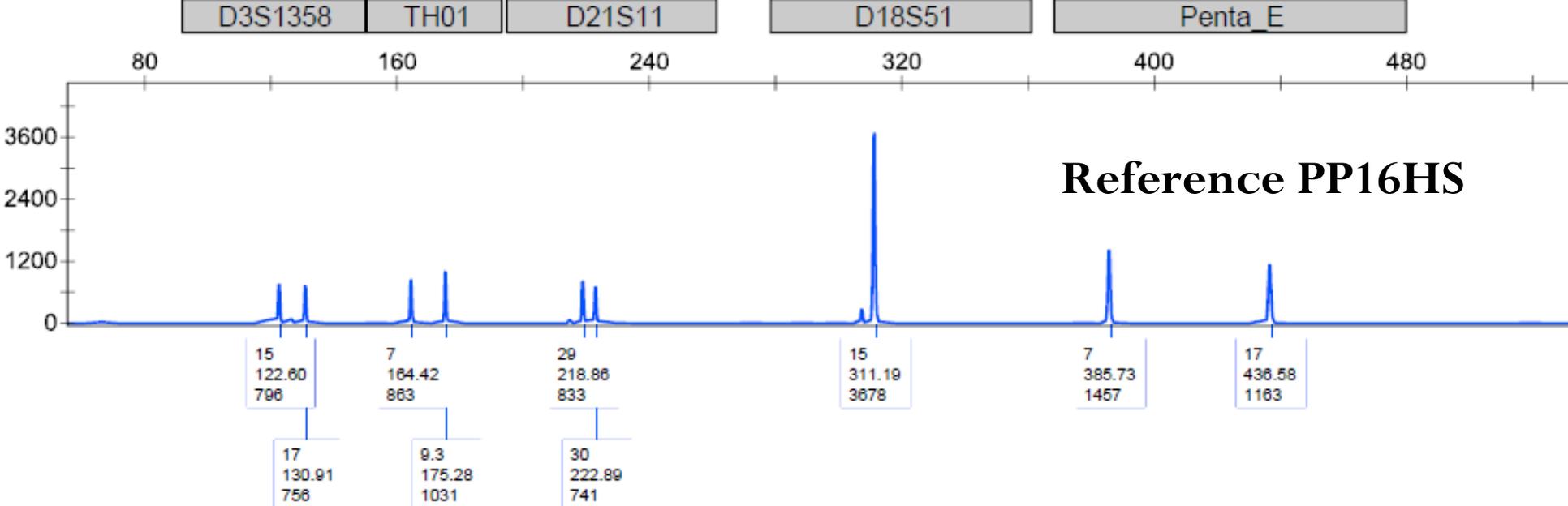


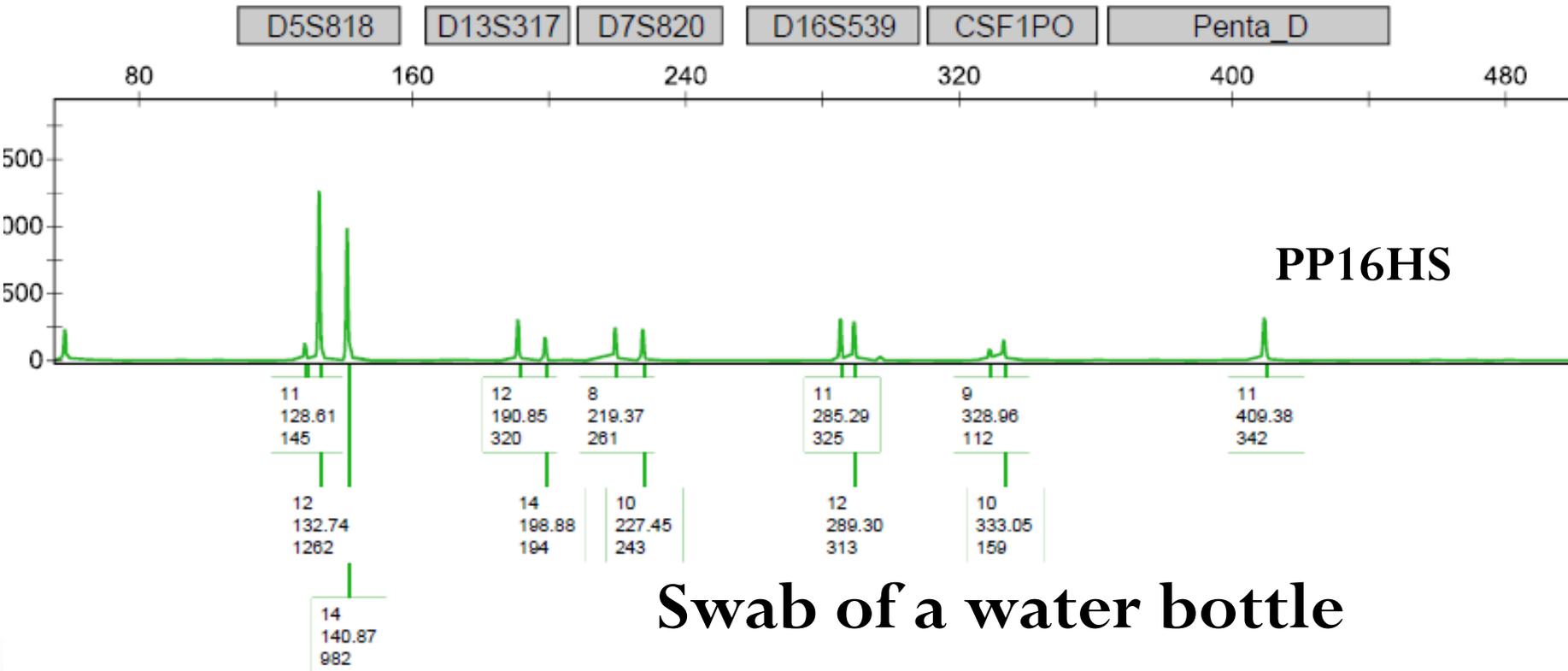
Description	Quantification Value
Left Hand Fingernail Scrapings	0.0304
Right Hand Fingernail Scrapings	0.1650
swabbings from gray hat	0.0074
swab of piece of glove	0.0702
swab of water bottle	0.3330
swab of Miller Lite bottle	0.0118
swab of hat	1.0500
swab of Gatorade bottle	0.0077
swab of Stanley tool	0.0071
swab of glove (under TV)	0.5710
swab of glove (parking lot)	1.8300
Cigarette Butt	0.1210
Cigarette Butt	0.0916
Leather Apron - Lower Left	0.3520
Leather Apron - Lower Left Corner	0.1330
Leather Apron - Upper Center	0.0064
swab from inside stairs	1.1900
cigarette	2.4200
blood from jewelry box	0.5510
blood from kitchen table	0.2680
blood from glass	0.1160
cigarette butt (8A)	0.5890
cigarette butt (CPeak-1)	4.4800
blood from air bag	0.1400
blood on gift card sleeve	0.1150

Description	Quantification Value
swab of cords	0.1730
swab of bottle	0.5870
swab of sweatshirt	0.5490
swab of mouth of water bottle	3.2800
blood from camp fuel can	0.7290
envelope cutting	0.3710
blood from glass	0.5560
suspected flesh	5.1500
cigarette butt	2.9100
blood from POE	0.4110
blood from lottery slip	0.1550
blood from broken window	0.6800
blood from computer bag	0.1840
blood from broken window	0.3430
blood from floor	4.0500

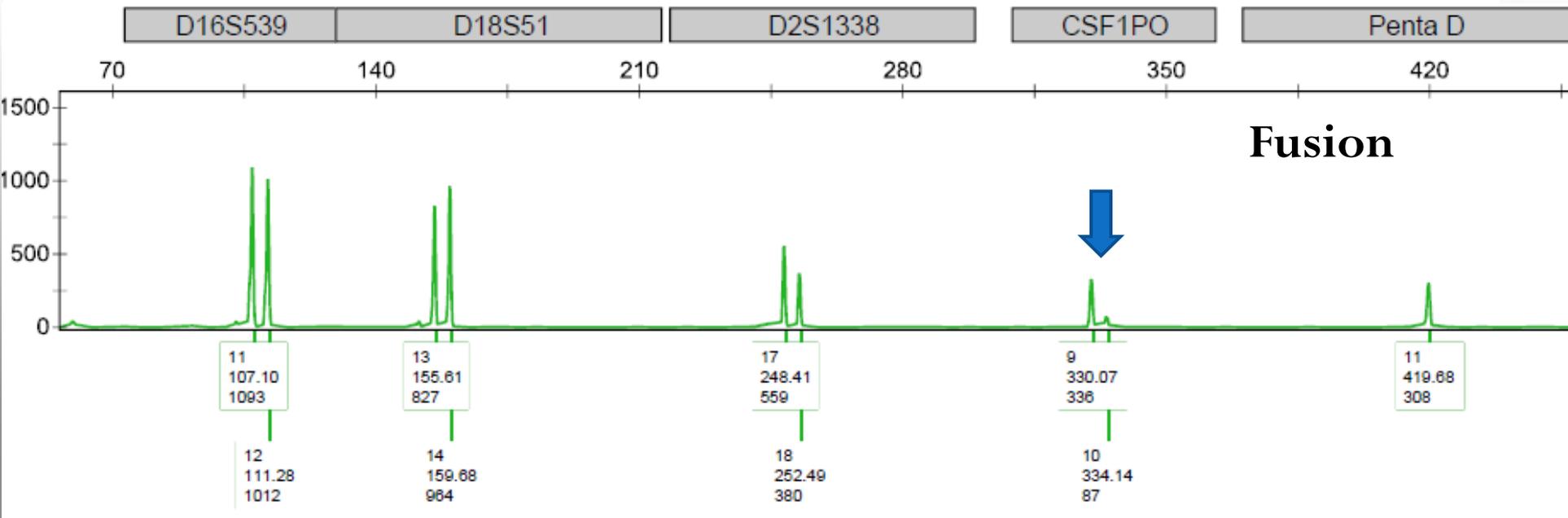
Description	Quantification Value	Y Quant
Vaginal / Cervical - EF	1.1200	0.0075
Anal / Rectal - EF	1.3400	0.0250
Vaginal / Cervical - EF	1.1900	N/A
Vaginal - EF	0.6990	0.0940
Vaginal - EF	1.0900	N/A
Vaginal / Cervical - SF	2.3900	5.0700
Anal / Rectal - SF	0.1570	0.3490
Vaginal / Cervical - SF	10.3000	0.0080
Vaginal - SF	9.3700	9.3800
Vaginal - SF	6.8100	0.0346







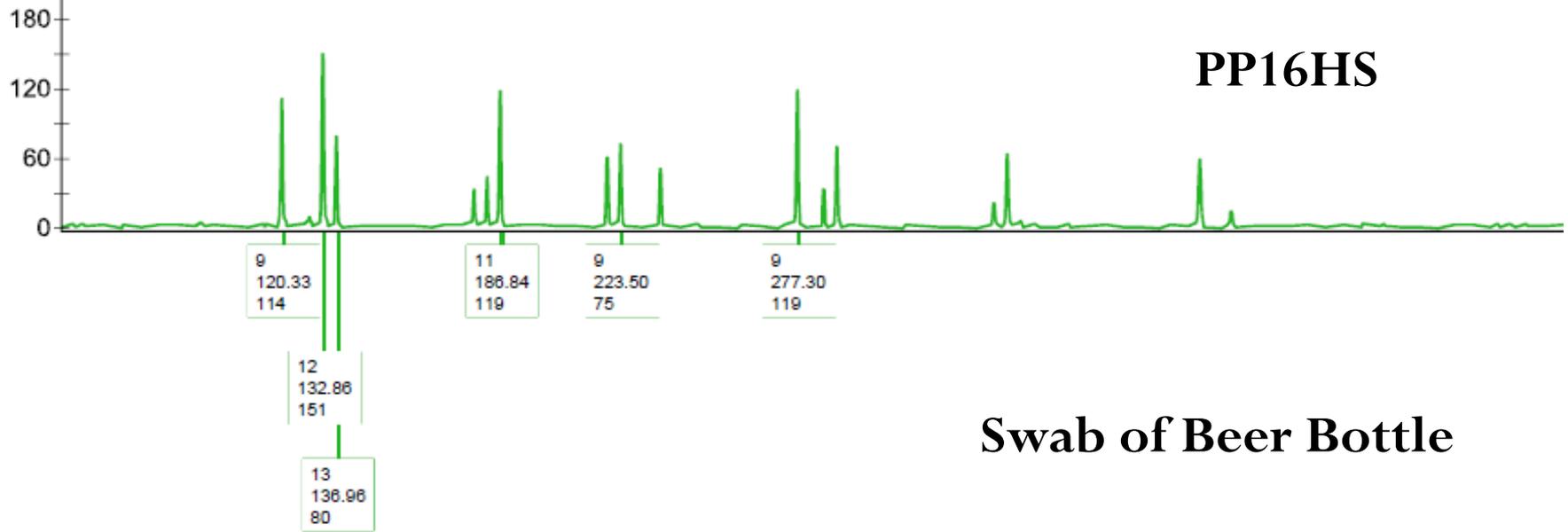
## Swab of a water bottle



D5S818 D13S317 D7S820 D16S539 CSF1PO Penta\_D

80 160 240 320 400 480

PP16HS

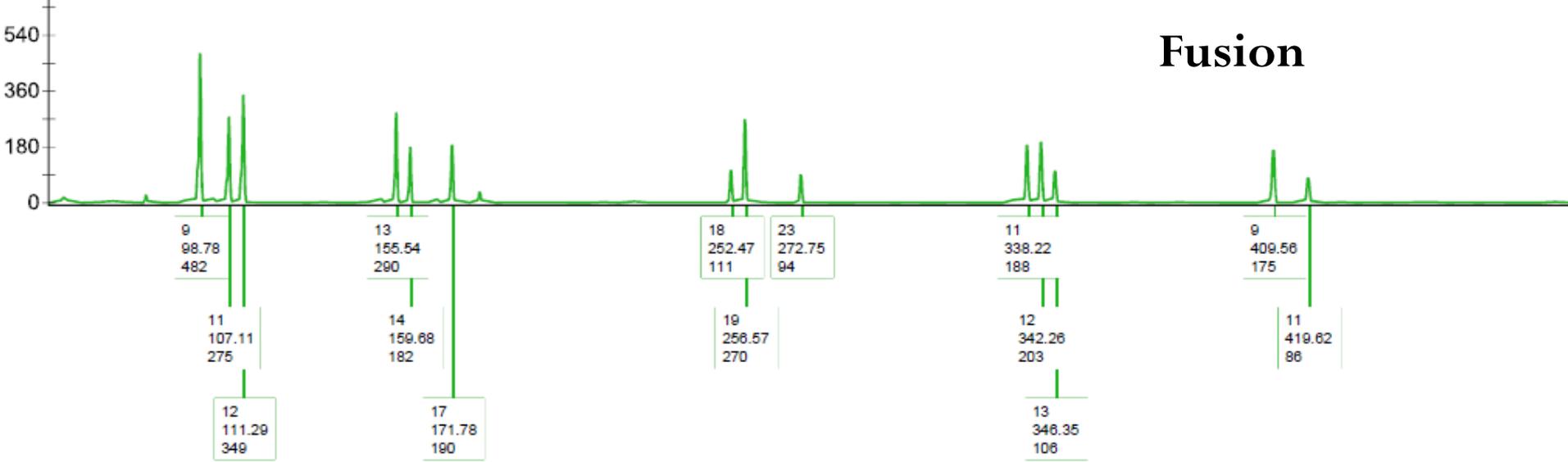


Swab of Beer Bottle

D16S539 D18S51 D2S1338 CSF1PO Penta D

70 140 210 280 350 420 490

Fusion



D3S1358

TH01

D21S11

D18S51

Penta\_E

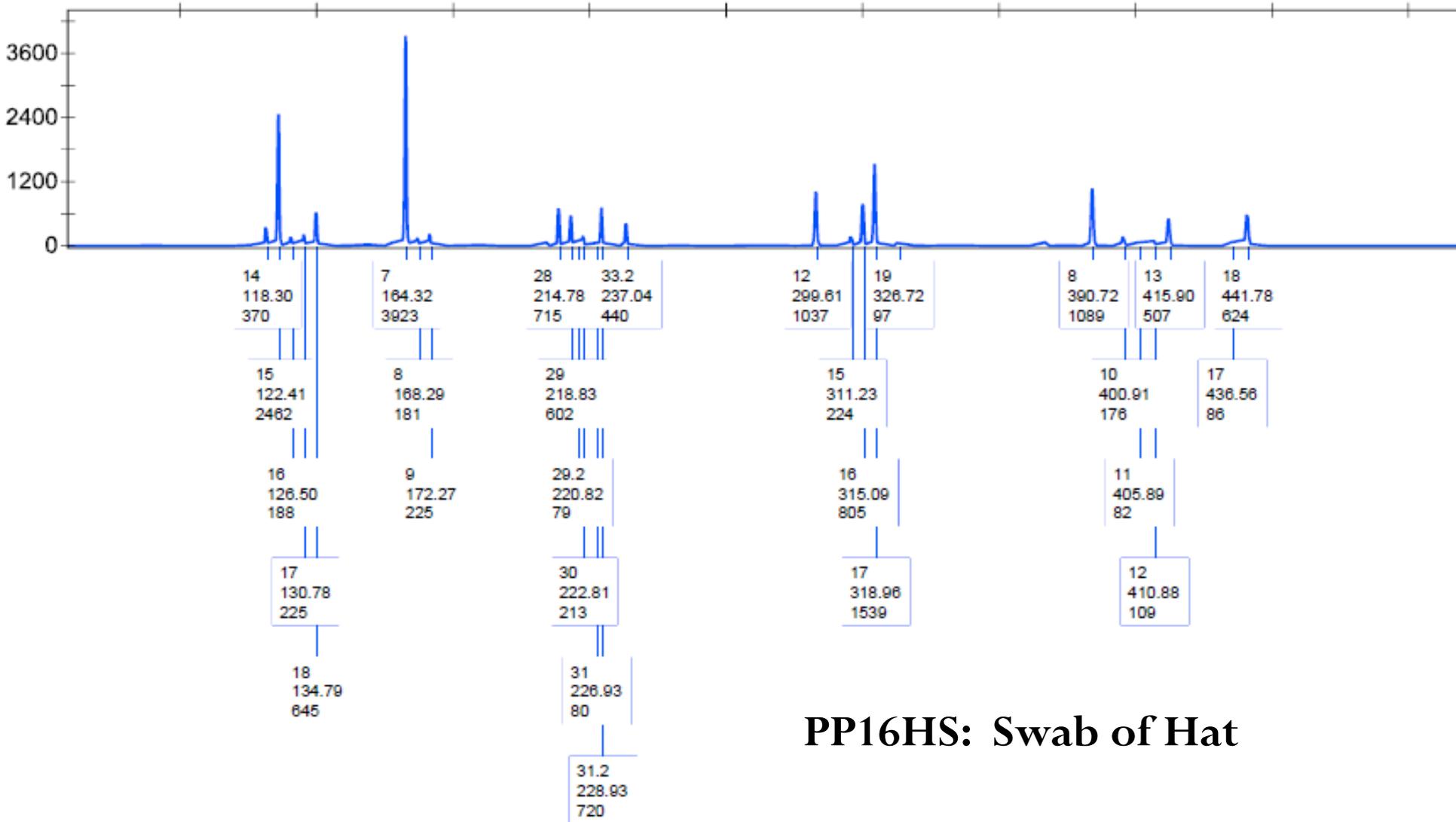
90

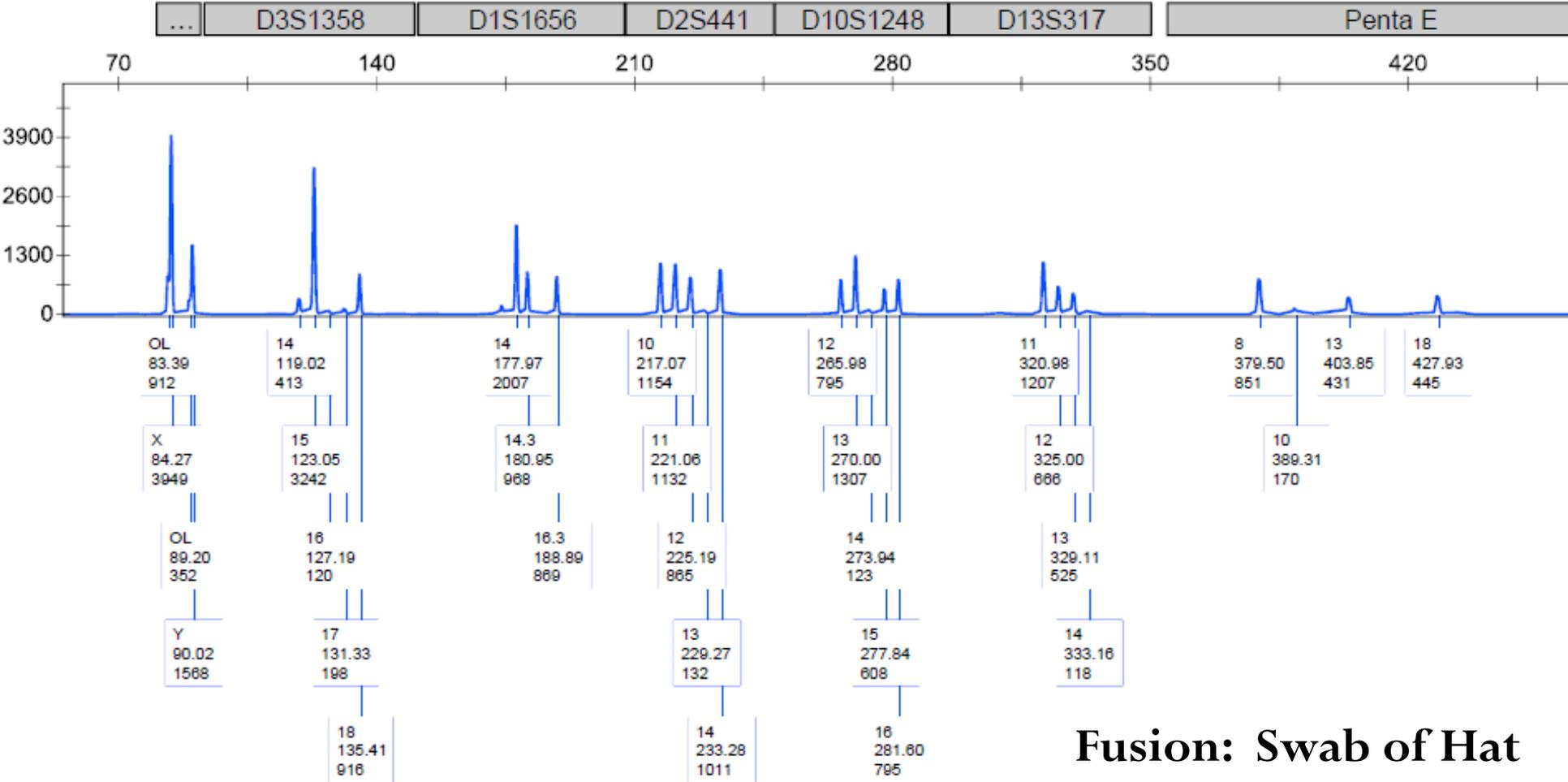
180

270

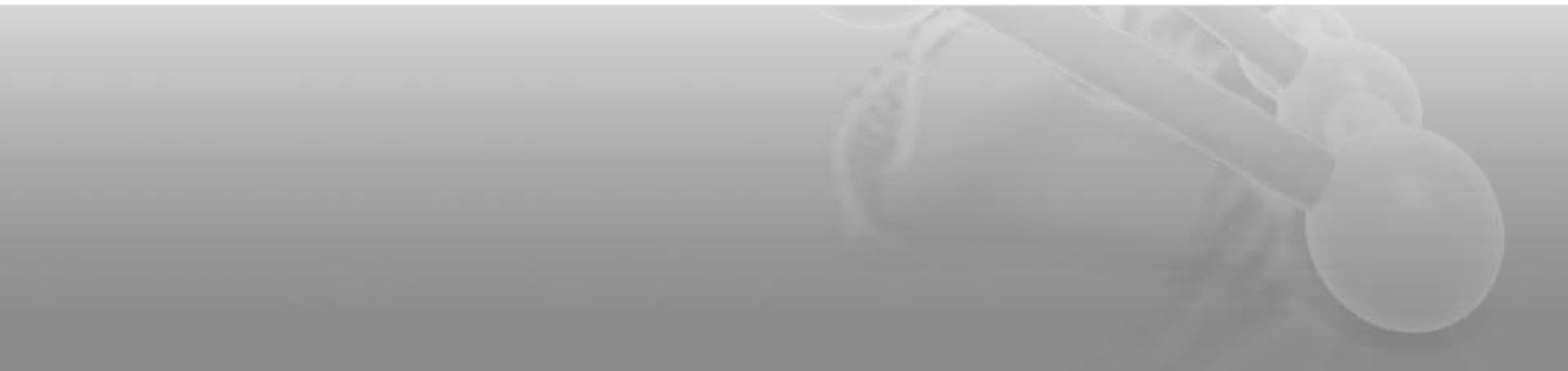
360

450



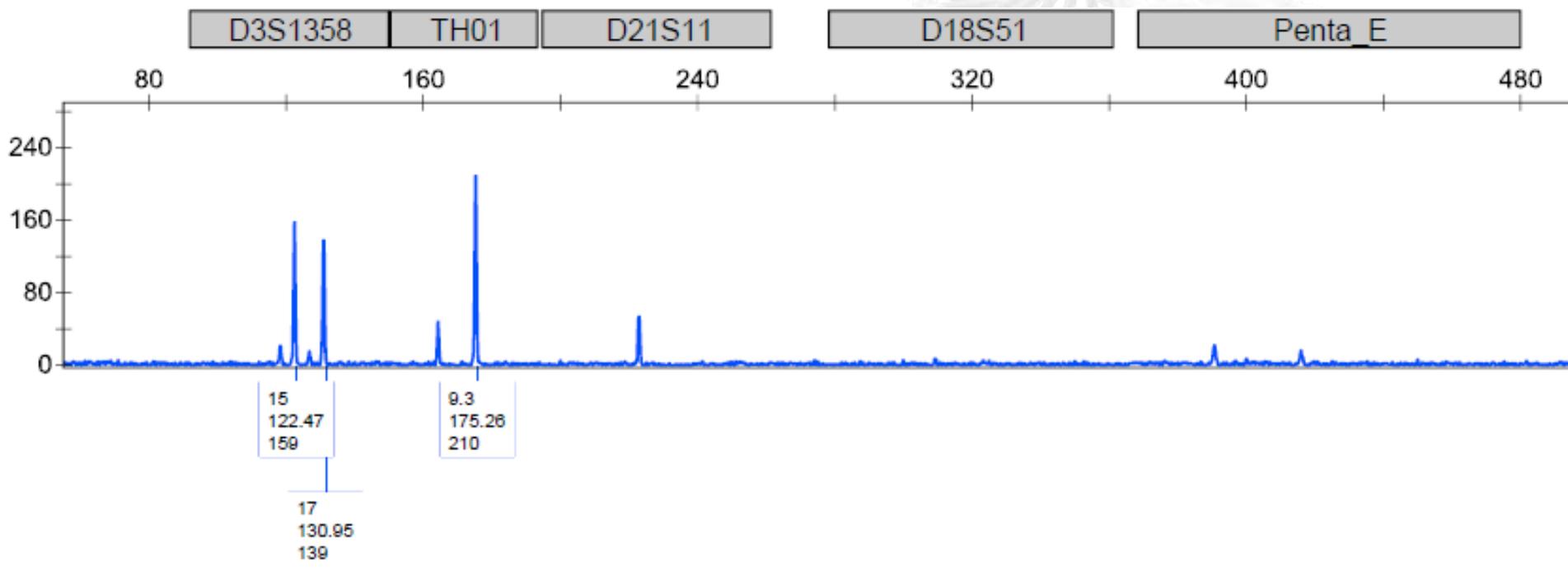
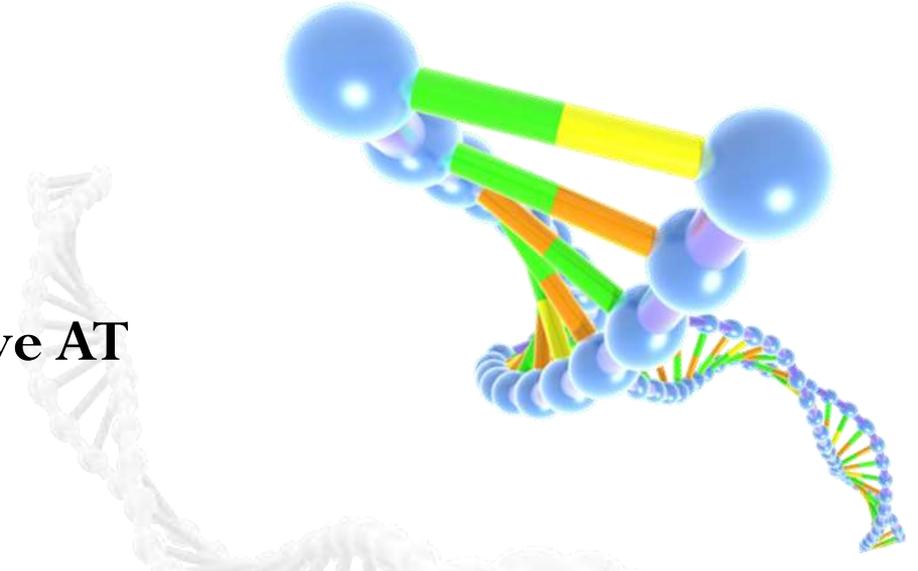


**Fusion: Swab of Hat**



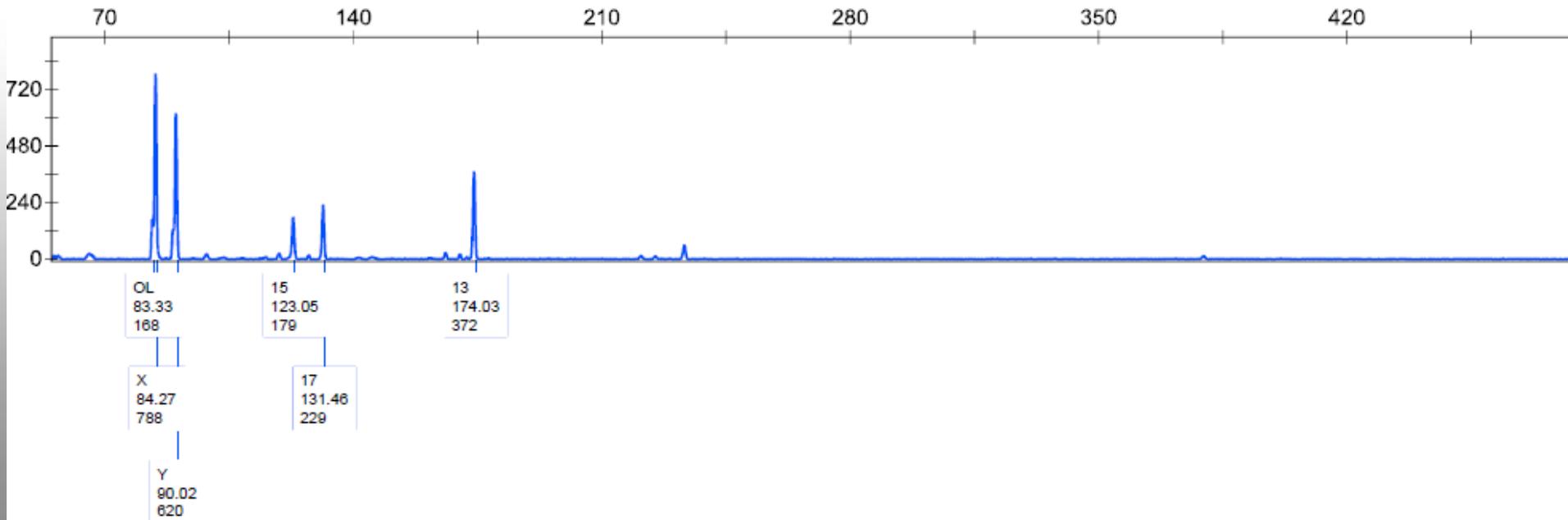
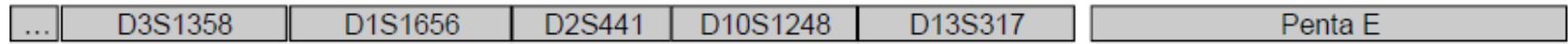
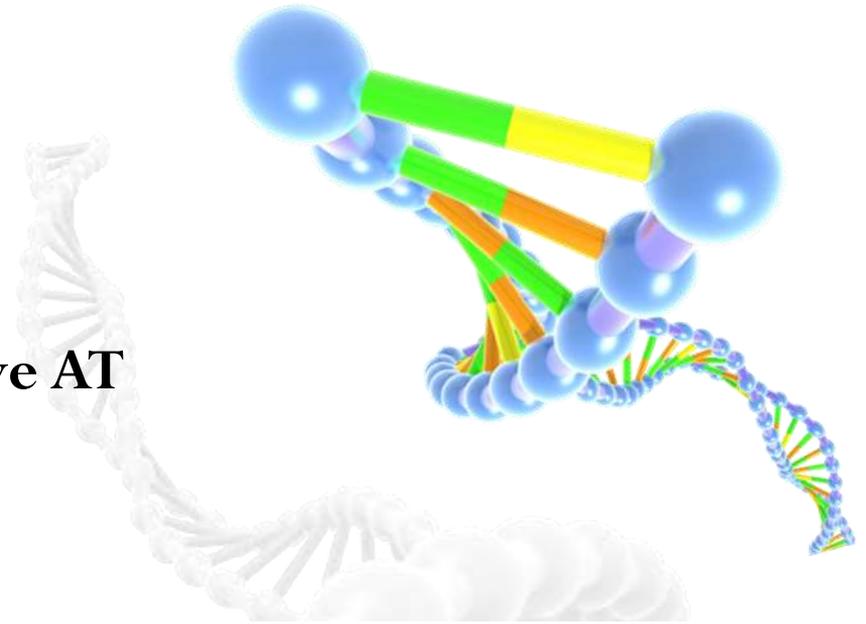
# PP16HS: Fingernail Scrapings

5 total markers with data above AT



# Fusion: Fingernail Scrapings

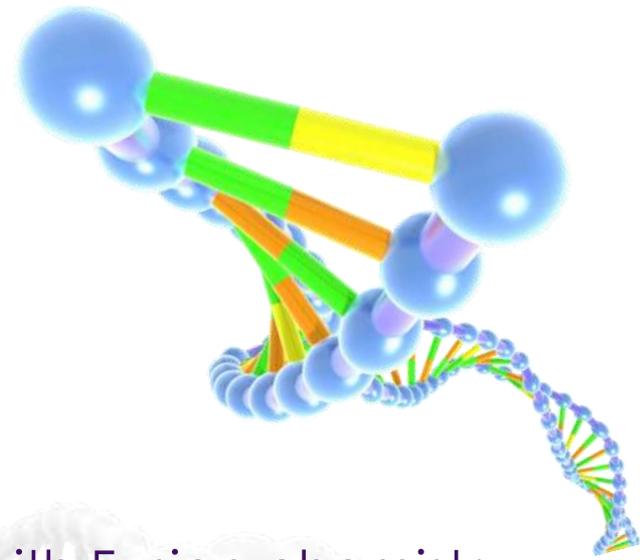
10 total markers with data above AT



# • INTERNAL VALIDATION

- NIST Standards Concordance

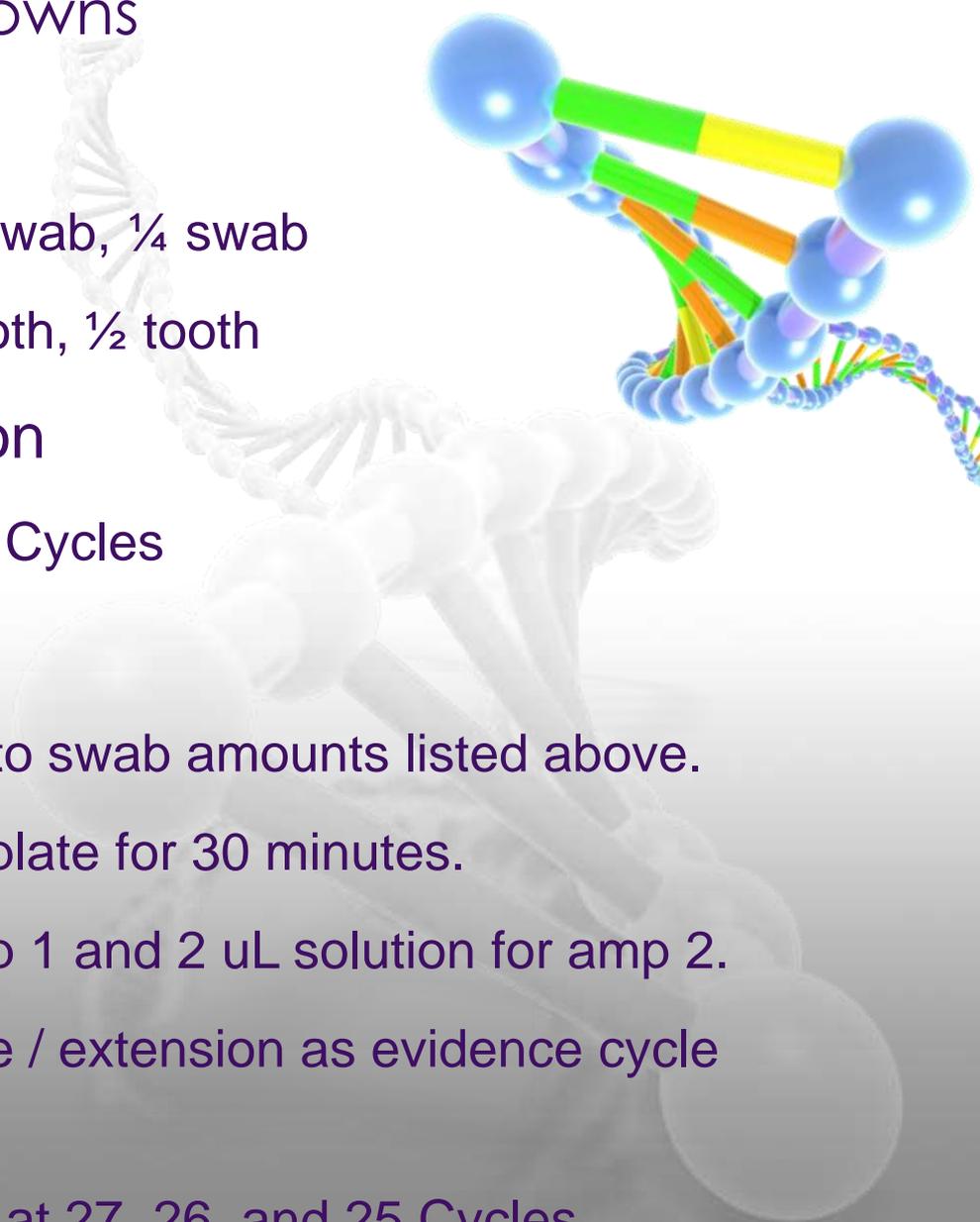
- NIST sample set 2931b was amplified with Fusion chemistry.
- All profiles obtained were concordant with the published profiles for the various components provided in the kit.



# • INTERNAL VALIDATION

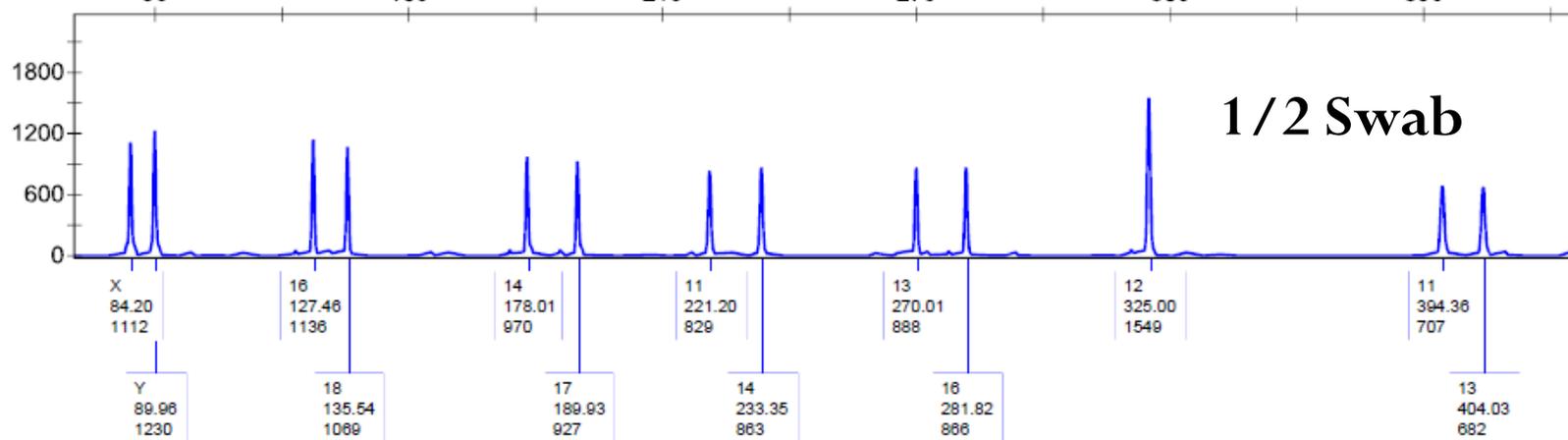
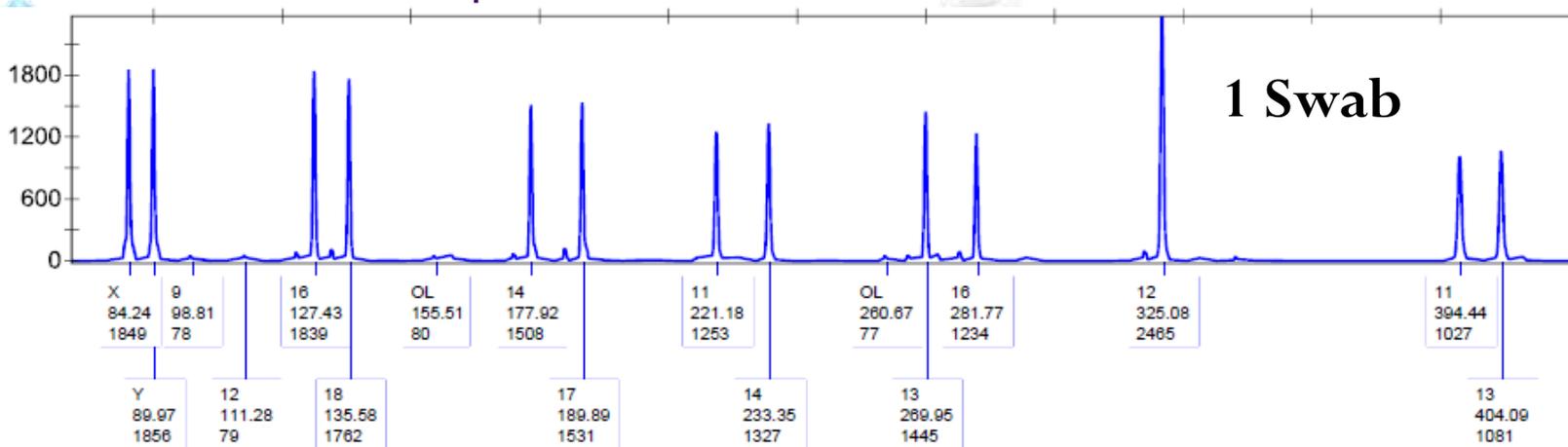
## Direct Amplification of Knowns

- Vary amount of swab
  - Cotton Swab – 1 swab,  $\frac{1}{2}$  swab,  $\frac{1}{4}$  swab
  - Omni Swab – 2 teeth, 1 tooth,  $\frac{1}{2}$  tooth
- Cycle Number Optimization
  - 30 Cycles / 27 Cycles / 25 Cycles
- Procedure:
  - Add 1 mL of swab solution to swab amounts listed above.
  - Place samples in 70 C hot plate for 30 minutes.
  - Use 1 uL of solution for amp 1 and 2 uL solution for amp 2.
  - Amp samples at same cycle / extension as evidence cycle parameters. (30 cycles)
  - Additional Amps performed at 27, 26, and 25 Cycles



# INTERNAL VALIDATION

## Direct Amplification of Knowns



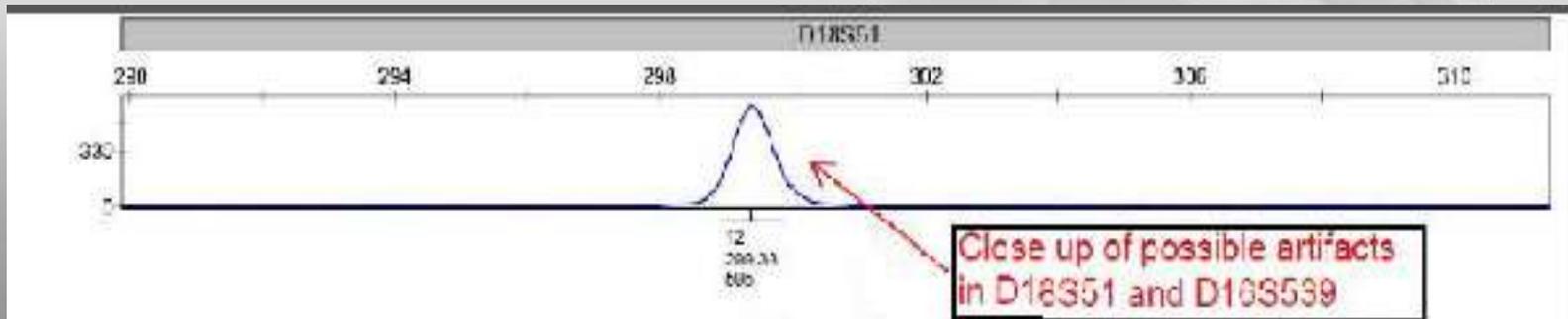
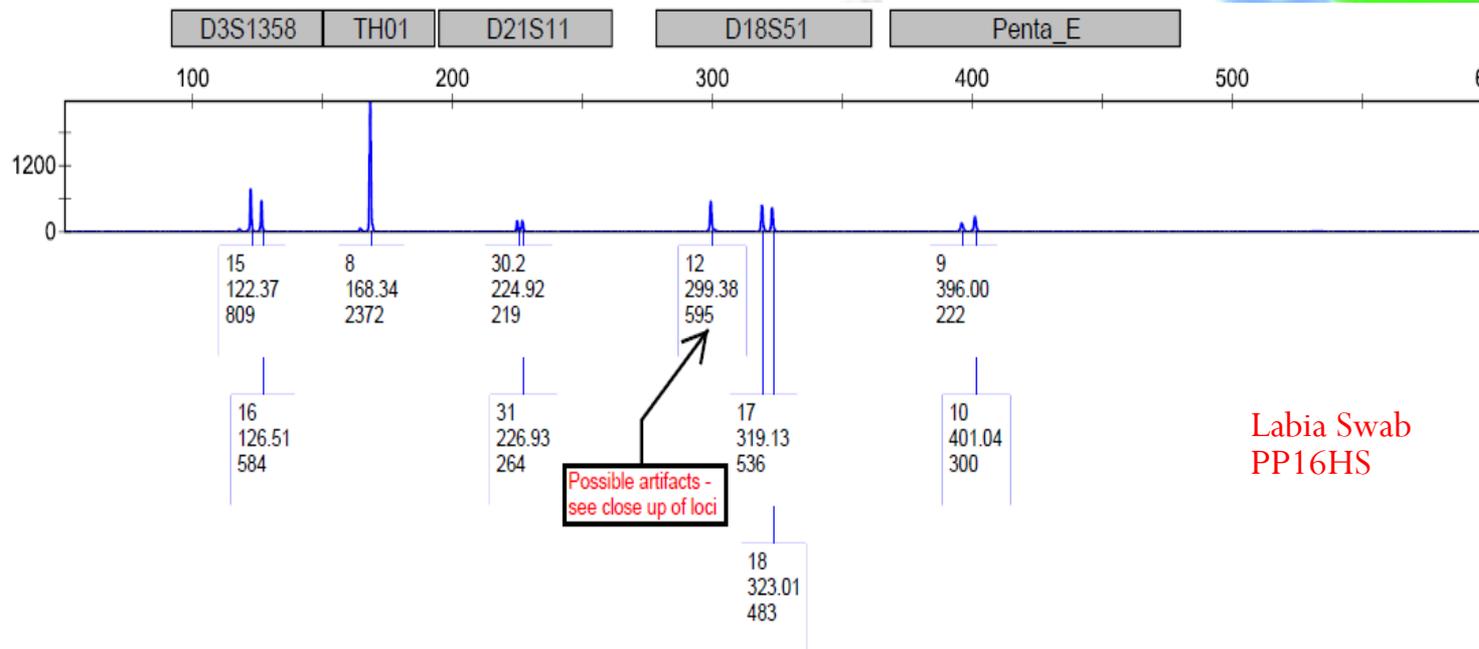


# Artifact Assessments



# • Additional Studies

- Comparison of artifacts



# Additional Studies

Comparison of artifacts

D18 WITH PP16HS

D18S51

300

12  
299.38  
595

17  
319.13  
536

18  
323.01  
483

artifacts -  
up of loci

D18 WITH FUSION

D18S51

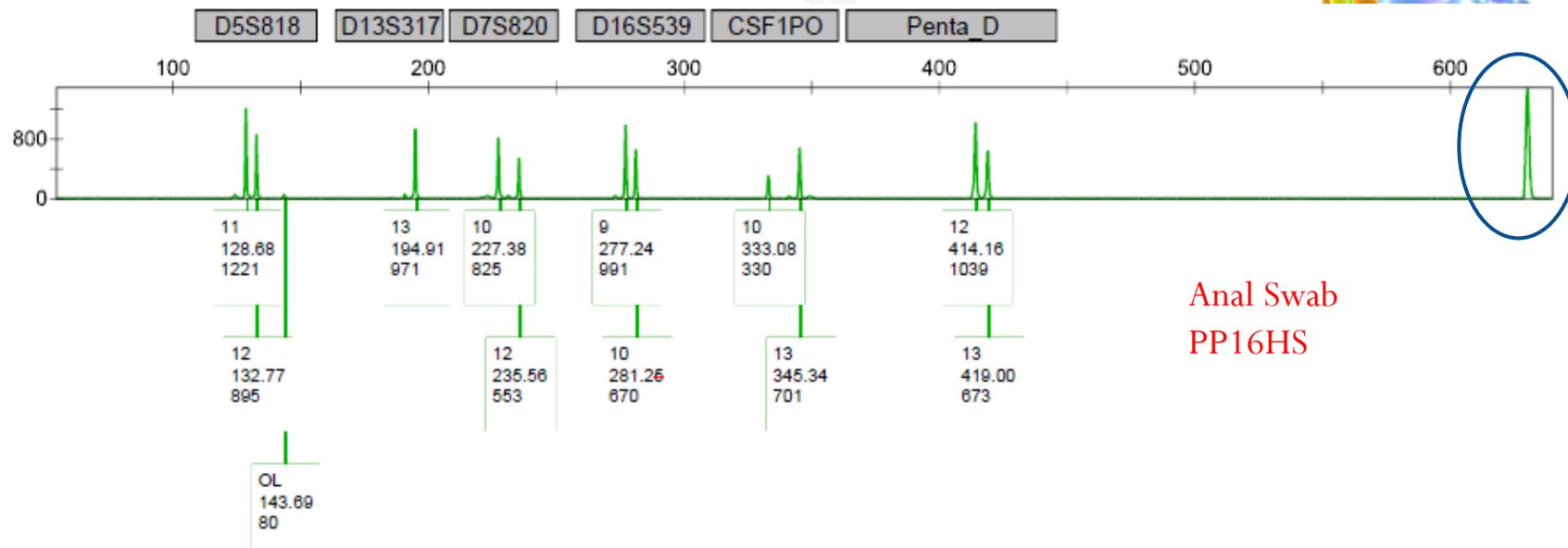
165

17  
607  
171.63

18  
550  
175.66

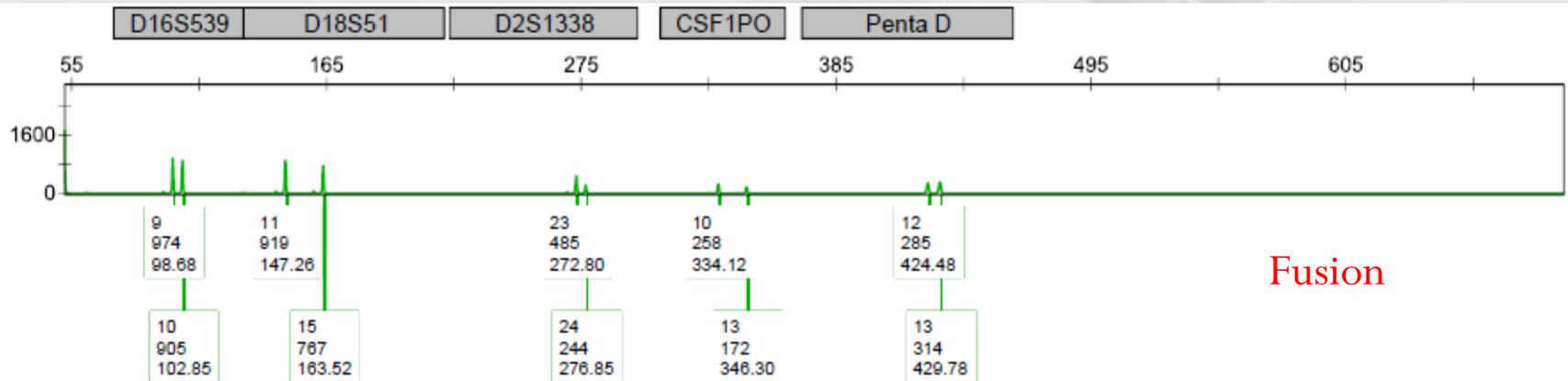
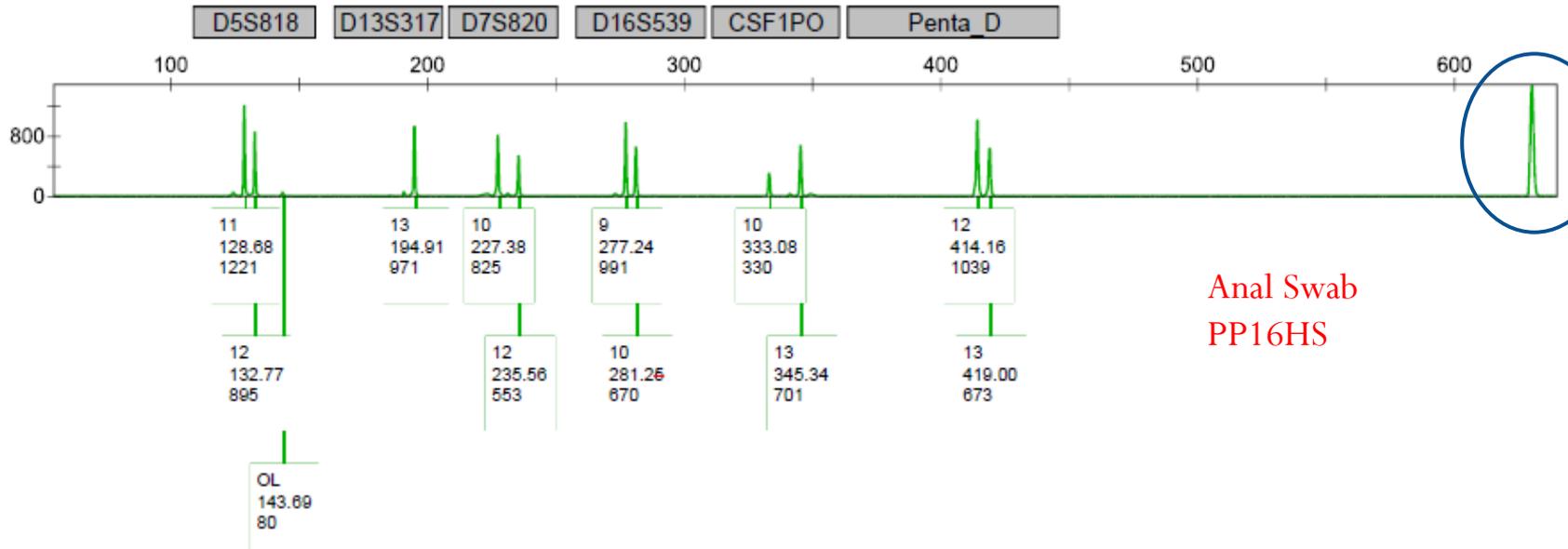
# • Additional Studies

- Comparison of artifacts



# • Additional Studies

- Comparison of artifacts



# •Database validation

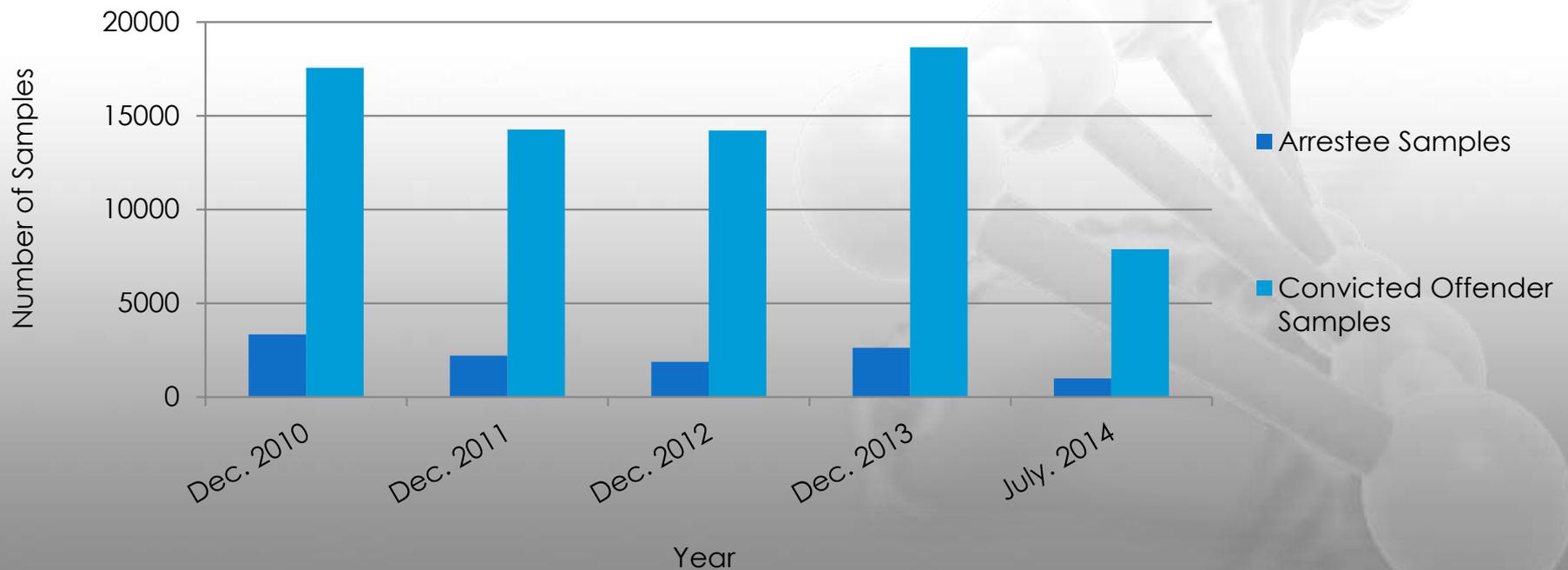
- Recently completed
- Being reviewed this week



# •Background on the MSP CODIS Unit

- Upload, on average, about 20,000 samples per year into the CODIS database.

**CODIS Database Samples Uploaded Annually**



# •Background on the MSP CODIS Unit

## cont.

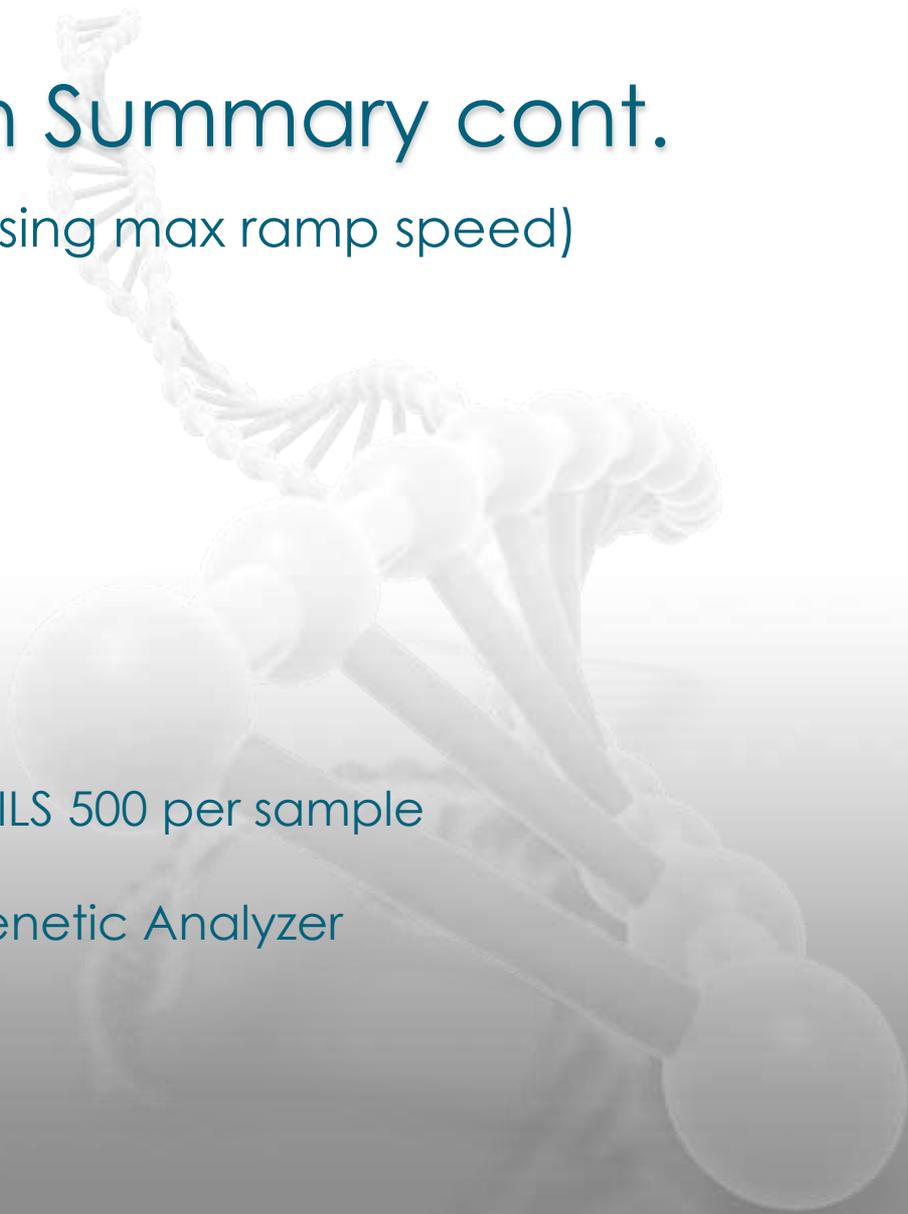
- Areas for improvement
  - Cost per sample to run with PowerPlex® Fusion
    - \$21.50 using the standard 25uL (1) amplification volume
    - \$8.60 using a reduced amplification volume (10uL)
      - Approximate yearly saving of \$208,673.63!
  - Processing time
    - Current high throughput process is about 14.5-15 hours
    - High throughput process in validation is about 7.5-8 hours

# •Background on the MSP CODIS Unit

## cont.

- Areas for improvement
  - Cost per sample to run with PowerPlex® Fusion
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    - High throughput process in validation is about 7.5-8 hours

# Pre-Validation Summary cont.



- Thermal cycling parameters (using max ramp speed)
  - Initial Hold-
    - 96° C for 1 min
  - Cycles (25, 26 or 29)
    - 94° C for 10sec
    - 59° C for 1min
    - 72° C for 30sec
  - Soak
    - 60° C for 40min
    - 4° C for ∞
- Post-PCR conditions
  - 10uL HiDi formamide and 1uL ILS 500 per sample
  - 1uL allelic ladder
  - Injection times on the 3500 Genetic Analyzer
    - 10sec- primary injection time
    - 6sec- oversaturated samples
    - 18sec- samples with low RFUs

# •Validation: Detection Thresholds

## • Results- Analytical Threshold (AT)

Analytical Threshold (AT) Calculations using 26 cycles (19 positives analyzed)							
Dye Color	Average (RFUs)	Standard Deviation	# Data Points	3x stdev (LOD)	Adjusted Average (3x RFUs)	10x stdev (LOQ)	Adjusted Average (10x RFUs)
Blue	5	7.23	5536	22	27	72	77
Green	8	6.20	5675	19	27	62	70
Yellow	11	7.81	6188	23	34	78	89
Red	12	7.29	6433	22	34	73	85

About 100 RFUs.  $1.5 \times 100 = 150$ . Use 150 for AT

## • Results- Peak declaration in Orange Dye channel

Peak Threshold calculations for ILS (Orange dye channel)- 25 and 26 cycle						
Average Height (RFUs)	Standard Deviation	3x stdev	10x stdev	Method 1 (LOD) (RFUs)	Method 4 (LOQ) (RFUs)	# Data Points
3	4	11	35	14	38	7362

About 40 RFUs.  $1.5 \times 40 = 60$ . Use **60** as peak cut-off value for **ILS (Orange dye channel)**

# •Validation: Detection Thresholds

## • Results- Stochastic Threshold (ST)

Statistical Threshold (ST) Determination (12 samples examined)

Cycle Number	[DNA]	Sample Name	Marker	Allele 1	Allele 2	Height 1	Height 2	
25	0.5	2800M-0.5ng-Rep1-10uL-25cycle	D12S391	18	18	159	159	
	0.3	2800M-0.3ng-Rep2-10uL-25cycle	D12S391	18	18	157	157	
	0.2		2800M-0.2ng-Rep1-10uL-25cycle	D13S317	9	9	159	159
			2800M-0.2ng-Rep1-10uL-25cycle	D1S1656	13	13	168	168
			2800M-0.2ng-Rep1-10uL-25cycle	Penta D	12	12	155	155
			2800M-0.2ng-Rep2-10uL-25cycle	D10S1248	15	15	178	178
			2800M-0.2ng-Rep2-10uL-25cycle	D13S317	9	9	180	180
			2800M-0.2ng-Rep2-10uL-25cycle	D2S441	14	14	170	170
			2800M-0.2ng-Rep2-10uL-25cycle	FGA	20	20	163	163
			2800M-0.2ng-Rep2-10uL-25cycle	Penta E	7	7	169	169
			0.15		2800M-0.15ng-Rep1-10uL-25cycle	D1S1656	12	12
	2800M-0.15ng-Rep1-10uL-25cycle	Penta D			13	13	173	173
	2800M-0.15ng-Rep2-10uL-25cycle	D1S1656			12	12	164	164
	2800M-0.15ng-Rep2-10uL-25cycle	D2S441			14	14	169	169
	2800M-0.15ng-Rep2-10uL-25cycle	FGA			20	20	168	168
2800M-0.15ng-Rep2-10uL-25cycle	Penta E	7			7	161	161	
26	0.2	2800M-0.2ng-Rep1-10uL-26cycle	D12S391	23	23	159	159	
	0.1	2800M-0.1ng-Rep3-10uL-26cycle	D2S1338	25	25	195	195	
		2800M-0.1ng-Rep1-10uL-26cycle	D8S1179	14	14	170	170	

highest peak is 195 (about 200).  $1.5 \times 200 = 300$ . ST will be 300

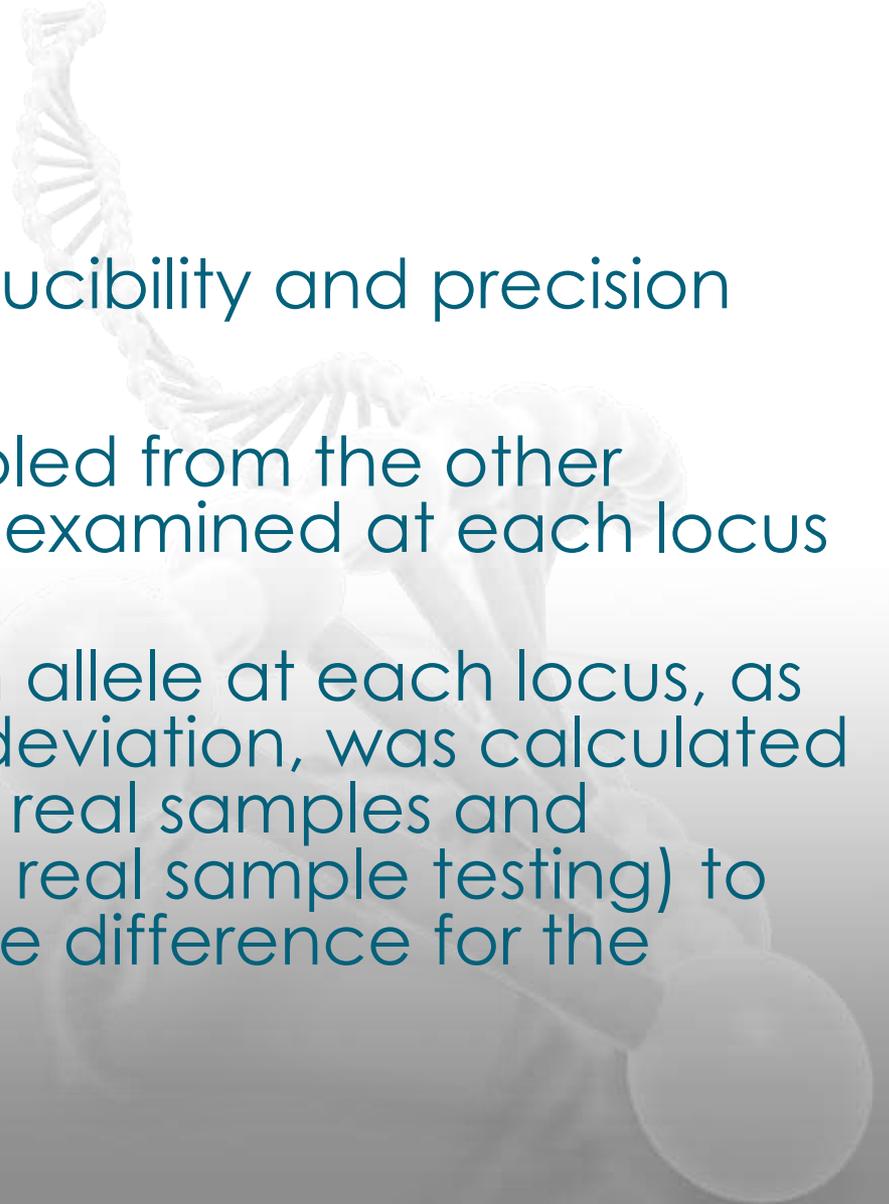
# •Validation: Reproducibility and Precision

## • Goal

- Determine the reproducibility and precision

## • Method

- 16 allelic ladders (pooled from the other validation tests) were examined at each locus for consistency.
- The average for each allele at each locus, as well as the standard deviation, was calculated then compared to 12 real samples and controls (pooled from real sample testing) to determine percent size difference for the given alleles.



# •Validation: Reproducibility and Precision

## • Results

### • Reproducibility

- 5872 data points were examined in the allelic ladder calculations.
- The maximum standard deviation was 0.09 at Penta D, allele 16
- The minimum standard deviation was 0.02 at various loci and alleles.

### • Precision

- 576 data points were examined in the comparison
- The minimum percent size difference was 0% at various loci and alleles.
- The maximum percent size difference was 28.5% at D12S391, allele 22
  - Corresponded to a base pair (bp) size difference of 0.479.

## • Conclusion

- The data from the allelic ladder calculations demonstrate that the Powerplex® Fusion amplification kit is reproducible.
- When compared to real samples and controls it also demonstrates that the precision falls within the acceptable range of 0.5 bp.

# •Validation: Sensitivity

## • Results

### • Punch Protocol

#### • Initial Testing:

- The 10 second and 18 second injection times @25 cycle both experienced allelic dropout at 0.5ng.
- The 10 second and 18 second injection times @26 cycle both experienced allelic dropout at 0.1 ng.

#### • Expanded Testing:

##### • 25 cycle

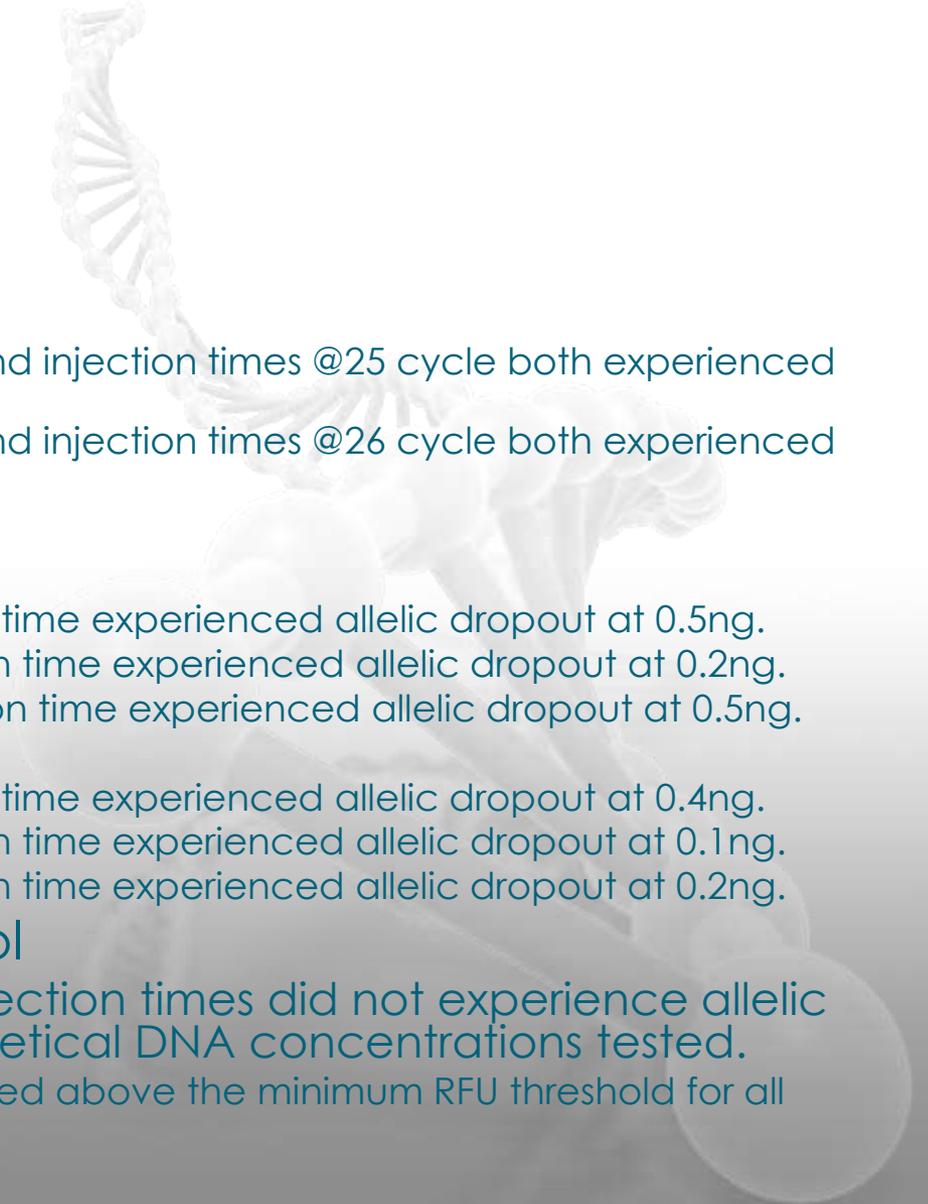
- The 6 second injection time experienced allelic dropout at 0.5ng.
- The 10 second injection time experienced allelic dropout at 0.2ng.
- The 18\* second injection time experienced allelic dropout at 0.5ng.

##### • 26 cycle

- The 6 second injection time experienced allelic dropout at 0.4ng.
- The 10 second injection time experienced allelic dropout at 0.1 ng.
- The 18 second injection time experienced allelic dropout at 0.2ng.

### • Manual Extraction Protocol

- The 6, 10 and 18 second injection times did not experience allelic dropout at any of the theoretical DNA concentrations tested.
  - Correct profiles were obtained above the minimum RFU threshold for all samples tested.



# •Validation: Sensitivity

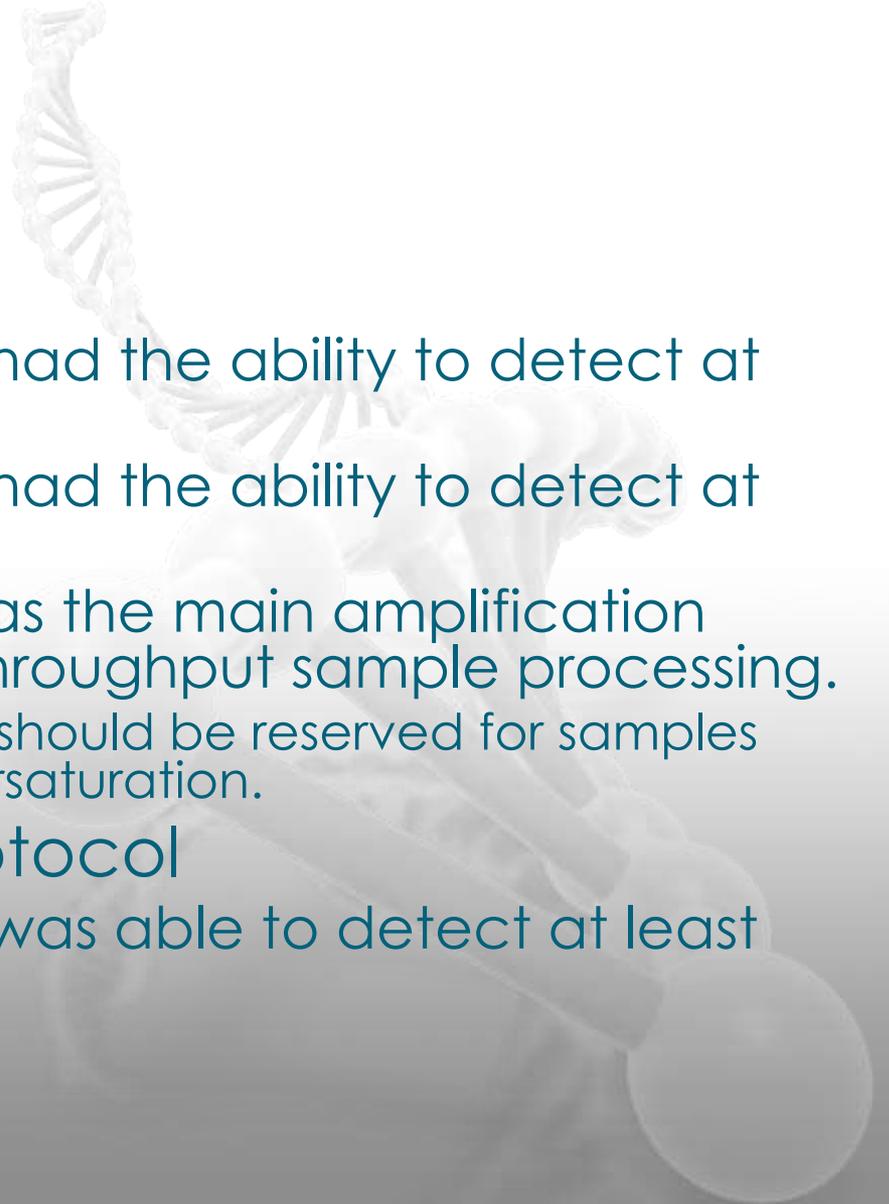
## • Conclusion

### • Punch Protocol

- The 25 cycle protocol had the ability to detect at least 0.3ng of DNA.
- The 26 cycle protocol had the ability to detect at least 0.15ng of DNA.
- 26 cycles will be used as the main amplification protocol for the high throughput sample processing.
  - The 25 cycle protocol should be reserved for samples that experienced oversaturation.

### • Manual Extraction Protocol

- The 29 cycle protocol was able to detect at least 0.1ng of DNA



# •Validation: Contamination Assessment

## •Method- Contamination Lists

Laboratory Substances
Inhibitor Type
Bleach (BL)
TE <sup>-4</sup> (TE)
Envirocide (EN)
Fingerprint Ink (INK)

Ingestible Substances Labeling Nomenclature		
Inhibitor Type	ID Number	Sub-category
Food Dyes (FD)	1= Freeze Pops	P= Purple
		G= Green
		B= Blue
		R= Red
		Y= Yellow
		O= Orange
	12= Hard Candy Lollipops- Brand 1	RW= Red Watermelon
		P= Purple
		RC= Red Cherry
		G= Green
13= Hard Candy Lollipops- Brand 2	RS= Red Strawberry	
	B= Blue	
	BR= Brown Root beer	
Sugar (SG)	2= Cookie Sandwiches	G= Golden
		C= Chocolate
	3= Chocolate (solid candy)	
Salt (ST)	4= Cheese coated snack	
	5= Sports drink	Y= Yellow W= White
	6= Sunflower Seeds	
Caffeine (CF)	7= Coffee	
	8= Tea	
Breath fresheners (BF)	9= Breath mint	
	10= Mouth wash	

# Validation: Contamination Assessment

- Results- Part 1- Ingestible Substances

- Food Dyes: 5 samples failed to generate a complete profile
  - On average, FD12RS and FD1R generated lower RFUS
  - FDY and FD1P, on average, generated normal to higher RFUs
  - In addition, FD12P, FD12RC and FD13BR generated lower RFUs, but obtained complete profiles

Ingestible Substances Labeling Nomenclature		
Inhibitor Type	ID Number	Sub-category
Food Dyes (FD)	1= Freeze Pops	<b>P= Purple</b>
		G= Green
		B= Blue
		<b>R= Red</b>
		<b>Y= Yellow</b>
		O= Orange
	12= Hard Candy Lollipops- Brand 1	RW= Red Watermelon
		<b>P= Purple</b>
		<b>RC= Red Cherry</b>
		G= Green
	13= Hard Candy Lollipops- Brand 2	<b>RS= Red Strawberry</b>
		B= Blue
		<b>BR= Brown Root beer</b>

# •Final Thoughts

- Powerplex® Fusion was found to be
  - Reliable and Precise with-in a 0.5bp window
  - Sensitive to 0.15ng and 0.1ng (punched and manually extracted samples respectively)
  - Real samples were consistent with prior allele calls
    - Only exceptions included AI's and the D8 locus in one sample
  - Contaminates, overall, did not pose a problem for profile generation
    - Only contaminate type with severe inhibition was the cheese coated snack.
- This new processing work flow and amplification kit should decrease the amount of sample processing time to about 8 hours.